universität wien

DISSERTATION

Titel der Dissertation

Molecular studies in Bromeliaceae:
Implications of plastid and nuclear DNA markers for phylogeny, biogeography, and character evolution with emphasis on a new classification of Tillandsioideae

Verfasser

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Wien, 2012

Studienkennzahl lt. Studienblatt:	A 091 438
Dissertationsgebiet lt. Studienblatt:	Botanik
Betreuerin:	Ao. Univ.-Prof. Dr. Rosabelle Samuel

Acknowledgements

Warm thanks go to my supervisor, Rosabelle Samuel, for her support and guidance through the years, and to Walter Till for his long-standing collaboration and sharing his taxonomic expertise on Bromeliaceae without which the research would not have been successful.

Thanks go to Tod F. Stuessy, emeritus head of the department of Systematic and Evolutionary Botany at the University of Vienna, for making the laboratory facilities available.

Above all, I would like to thank my colleagues from the molecular laboratory, Elfriede Grasserbauer, Verena Klejna and Gudrun Kohl, for their patience and support that enabled me to complete this work.

I would like to extend my gratitude to people who provided plant material, especially Renate Ehlers, Liselotte and Helmut Hromadnik, Elton M.C. Leme, Jose M. Manzanares, and Juan P. Pinzón.

Many thanks go to all co-authors, especially to Thomas J. Givnish, Georg Zizka and Katharina Schulte for helpful discussions, and also to Félix Forest and Mark W. Chase at the Royal Botanic Gardens, Kew, U.K., with whom I shared useful discussions on low-copy nuclear genes during my stay at the Jodrell Laboratory.

Special thanks go to Ovidiu Paun, Anton Russell, and Hermann Voglmayr for valuable discussions, critical review of some parts of this thesis and technical support.

Several members of the department of Systematic and Evolutionary Botany in Vienna have supported me intellectually and morally and this is gratefully acknowledged: Anne-Caroline Cosendai, Kathere Emadzade, Patricio López Sepúlveda, Eva Temsch, Barbara Turner, and former students, Cordula Blöch, Sutee Duangjai, Eva Maria Mayr, Carolin A. Rebernig, Stefan Safer, Reinhold Stockenhuber.

Finally I want to thank my family and all friends all over the world who have helped and supported me in various ways, and anybody else I may have forgotten to mention.

Financial support provided by the University of Vienna, the Commission for Interdisciplinary Ecological Studies (KIÖS, grant no. 2007-02) at the Austrian Academy of Sciences (ÖAW), the SYNTHESYS (Synthesis of Systematic Resources) program funded by European Union, and the Austrian Science Foundation (FWF; grant no. P13690) is greatly acknowledged.

Danksagung

Besonderer Dank geht an meine Betreuerin, Rosabelle Samuel, für ihre langjährige Unterstützung und Begleitung, und an Walter Till für seine fortwährende Zusammenarbeit und Einbringung seiner taxonomischen Bromelien-Expertise, ohne die diese Forschungsarbeit nicht erfolgreich gewesen wäre.

Dank geht an Tod F. Stuessy, emeritierter Leiter des Departments für Botanische Systematik und Evolutionsforschung der Universität Wien, für die Bereitstellung der Laboreinrichtungen.

Vor allem möchte ich meinen Kolleginnen aus dem Molekularlabor, Elfriede Grasserbauer, Verena Klejna und Gudrun Kohl, für ihre Geduld und Unterstützung danken, die es mir ermöglicht haben, diese Arbeit fertig zu stellen.

Herzlicher Dank geht an mehrere Personen, die Pflanzenmaterial zur Verfügung gestellt haben, insbesondere Renate Ehlers, Helmut und Liselotte Hromadnik, Elton M.C. Leme, Jose M. Manzanares, und Juan P. Pinzón.

Dank geht an alle Mitautoren, vor allem an Thomas J. Givnish, Georg Zizka und Katharina Schulte für hilfreiche Diskussionen und auch an Félix Forest und Mark W. Chase der „Royal Botanic Gardens, Kew“ (U.K.) für wertvolle Diskussionen über nukleäre Gene während meines Forschungsaufenthaltes im „Jodrell Laboratory“.

Besonderer Dank geht an Ovidiu Paun, Anton Russell und Hermann Voglmayr für wertvolle Diskussionen, kritische Überprüfung einiger Teile dieser Arbeit und technische Unterstützung.

Mehrere Kollegen des Departments für Botanische Systematik und Evolutionsforschung der Universität Wien haben mich intellektuell und moralisch unterstützt, wofür ich mich aufs Herzlichste bedanken möchte: Anne-Caroline Cosendai, Kathere Emadzade, Patricio López Sepúlveda, Eva Temsch, Barbara Turner; und ehemalige Studenten, Cordula Blöch, Sutee Duangjai, Eva Maria Mayr, Carolin A. Rebernig, Stefan Safer, Reinhold Stockenhuber.

Abschließend möchte ich meiner Familie und allen Freunden auf der ganzen Welt, die mir geholfen und mich in verschiedenster Weise unterstützt haben, und all jene ich vergessen habe zu erwähnen, danken.

Finanzielle Unterstützung wurde von der Universität Wien, der Kommission für Interdisziplinäre Ökologische Studien (KIÖS, Projektnr. 2007-02) an der Österreichischen Akademie der Wissenschaften (ÖAW), dem von der Europäischen Union geförderte SYNTHESYS-Programm („Synthesis of Systematic Resources“), und den Fonds zur Förderung der Wissenschaftlichen Forschung (FWF; Projektnr. P13690) bereit gestellt.

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Abstract

The monocot family Bromeliaceae comprises approximately 3,140 species distributed in tropical and subtropical regions of the New World from the southern United States to southern Argentina. The family is subdivided into eight subfamilies; the most species-rich are Tillandsioideae and Bromelioideae. Taxonomic concepts within Bromeliaceae are highly problematic, since discriminating morphological characters have been shown to be homoplastic or plesiomorphic. The present study aims to provide a robust phylogenetic framework for Bromeliaceae, especially for the most diverse and complicated subfamilies Bromelioideae and Tillandsioideae. Resulting phylogenies provide a basis to estimate the usefulness of morphological characters and to propose or strengthen hypotheses concerning evolutionary traits, biogeography, age and origins of bromeliads. The main questions raised are:

(1) Do additional sequence data from the plastid genome and a wider sampling within Bromeliaceae provide a better resolved, robust phylogenetic framework? What are the reasons for the low DNA sequence divergence observed up to now?
(2) Can nuclear DNA sequences be successfully implemented for phylogenetic reconstruction? What are the challenges to optimize nuclear markers and do they perform better than plastid loci?
(3) Can the resulting phylogeny based on plastid and nuclear DNA sequences together with the re-evaluated morphological characters provide a reasonable, stable classification?

To provide a more robust phylogenetic hypothesis for the classification of Bromeliaceae, eight rapidly evolving plastid DNA markers (*atpB-rbcL*, *matK*, *ndhF*, *psbA-trnH*, *rpl32-trnL*, *rps16*, *trnL* intron, *and trnL-trnF*) and 90 bromeliad species were included in the current study and analysed using maximum-parsimony, maximum-likelihood, and Bayesian approaches. Results support the formerly proposed eight-subfamily classification based on the single plastid gene *ndhF*. Support values for five of the subfamilies increase, but that for Lindmanioideae, Puyoideae, and Bromelioideae decrease as a result of expanded taxon sampling, including several more divergent species. The initially proposed monophyletic origin of Puyoideae cannot be unambiguously confirmed. Calibration of the resulting phylogeny against time and biogeographic analysis reveals that Bromeliaceae originated in the Guayana Shield about 100 million years ago (Ma) and spread radially into adjacent areas ca. 16—13 Ma. Extant lineages arose between 20 and 5 Ma. Andean uplift facilitated diversification of core Tillandsioideae about 14.2 Ma and Bromelioideae 10.1 Ma, the latter having their greatest diversity in the Brazilian Shield due to dispersal from the Andes. The most species-rich genera did not appear before 8.7 My with a high diversification between 5 and 4 Ma, which is most likely the reason for the comparatively low sequence divergence.

To test the usefulness of nuclear regions for phylogenetic reconstruction in Bromelioideae, DNA sequences of part of the low-copy nuclear gene phosphoribulokinase (*PRK*) and five

plastid loci (*matK*, 3'*trnK* intron, *trnL* intron, *trnL-trnF*, and *atpB-rbcL*) were investigated. Phylogenetic trees obtained from analyses of the *PRK* sequences do not contradict trees obtained from plastid markers. The *PRK* matrix shows a significantly higher number of potentially PICs (phylogenetically informative characters) than the plastid dataset (16.9% vs. 3.1%), which improves resolution and support in the resulting trees. Although *PRK* is not able to resolve relationships completely, the combined analysis with plastid markers yields good support for several uncertain relationships observed previously. The early diverging lineages can be identified ("basal bromelioids") and the remainder of the subfamily clustered into a highly supported clade ("eu-bromelioids"). Results indicate that taxonomic circumscriptions within "core bromeliads" are still insufficient, and relationships complex and difficult to solve. Several genera appear polyphyletic, and *Aechmea* as well as *Quesnelia* remain the most complicated genera of the subfamily. Most-parsimonious character state reconstructions for two evolutionary traits (tank habit, sepal symmetry) indicate that both characters have undergone few transitions within the subfamily and thus are not as homoplasious as previously assumed.

The comparative study of nuclear DNA sequences within Tillandsioideae shows that some nuclear markers are able to provide more information and a higher degree of resolution in phylogenetic trees than plastid markers. However, their utility does not depend only on sequence variability, but also on methodological challenges in using traditional Sanger-sequencing. The internal transcribed spacer of nuclear ribosomal DNA (ITS nrDNA) is not recommended as a suitable marker for phylogenetic investigations of Bromeliaceae due to the presence of strong secondary structures which create problems in amplification and sequencing as well as its low number of PICs for resolving deeper nodes. Amplified fragments of the genes malate synthase (*MS*) and RNA polymerase II, beta subunit (*RPB2*) are not helpful due to their small size and limited number of PICs. Glucose-6-phosphate isomerase (*PGIC*), nitrate reductase (*NIA*), and xanthine dehydrogenase (*XDH*) need to be further investigated. Phosphoribulokinase (*PRK*) and phytochrome C (*PHYC*) are useful nuclear markers and able to provide considerable resolution in phylogenetic trees, but some relationships are poorly supported.

The combined analysis of nuclear DNA sequence data (*PRK*, *PHYC*) with the already existing plastid DNA sequence data *(atpB-rbcL*, *matK*, *rbcL*, partial *rbcL-accD*, *rps16* intron, partial *trnK* intron, *trnL* intron and *trnL-trnF*) shows a significant increase of resolution within phylogenetic trees of Tillandsioideae. Nine accepted genera can be re-circumscribed and three new genera are described taxonomically based on morphology. For morphologically distinct species groups within *Racinaea* and *Tillandsia*, two new subgenera are erected. *Viridantha* has been downgraded to subgeneric rank. Poor sampling within the *Cipuropsis-Mezobromelia* clade and missing support for clades within *Tillandsia* prevent the recognition of further taxonomic groups.

Kurzfassung

Die Ananasgewächse (Bromeliaceae) sind mit etwa 3.140 Arten eine der artenreichsten Familien der einkeimblättrigen Blütenpflanzen, die in tropischen und subtropischen Regionen der Neuen Welt vom Süden der Vereinigten Staaten bis in den Süden Argentiniens vorkommen. Die Familie ist in acht Unterfamilien unterteilt, deren artenreichste die Tillandsioideae und Bromelioideae sind. Taxonomische Konzepte innerhalb der Bromeliaceae sind sehr problematisch, da zur Unterscheidung herangezogene morphologische Merkmale homoplastisch oder plesiomorph sind. Die vorliegende Studie zielt darauf ab, eine gut gestützte Stammbaumrekonstruktion für die Bromeliaceae zu erstellen, insbesondere für die komplexen Unterfamilien Bromelioideae und Tillandsioideae. Die resultierenden Phylogenien sollen eine Grundlage bieten, die taxonomische Verwendbarkeit von morphologischen Merkmalen zu bewerten und Hypothesen zur Merkmalsevolution, Biogeographie, Alter und Herkunft der Bromelien aufzustellen oder zu untermauern. Die wichtigsten Fragenstellungen sind:

(4) Zeigen eine breitere Probennahme innerhalb der Bromeliaceae sowie zusätzliche Sequenz-Daten aus dem Plastiden-Genom einen besser aufgelösten und gut gestützten Stammbaum? Was sind Gründe für die bisher beobachtete geringe DNS-Sequenz-Divergenz?
(5) Können nukleäre DNS-Sequenzen erfolgreich zur Erstellung von Stammbaumrekonstruktion verwendet werden? Was sind die Herausforderungen um nukleäre Marker zu optimieren und sind sie besser geeignet als Plastiden-Marker?
(6) Kann man mit Hilfe der auf Plastiden- und Kern-DNS-Sequenzen basierenden Phylogenie zusammen mit neu bewerteten morphologischen Merkmalen eine vernünftige, stabile Klassifikation erreichen?

Um eine besser abgestützte phylogenetische Hypothese für die Klassifizierung der Bromeliaceae zu erreichen, wurden in der aktuellen Studie acht schnell evoluierende Plastiden-DNS-Marker (*atpB-rbcL*, *matK*, *ndhF*, *psbA-trnH*, *rpl32-trnL*, *rps16*, *trnL* Intron und *trnL-trnF*) und 90 Bromelienarten ausgewählt und mit Maximum-Parsimony-, Maximum-Likelihood- und Bayesian-Methoden analysiert. Die Ergebnisse unterstützen die schon früher vorgeschlagene Einteilung in acht Unterfamilien, die auf einem einzigen Plastiden-Gen (*ndhF*) basiert. Unterstützungswerte für fünf der Unterfamilien haben sich erhöht, jedoch die für Lindmanioideae, Puyoideae und Bromelioideae sind infolge der erweiternden Taxon-Auswahl gesunken. Die ursprünglich vorgeschlagene Monophylie der Puyoideae kann nicht eindeutig bestätigt werden. Eine kalibrierte zeitliche Zuordnung der resultierenden Phylogenie und biogeographische Analysen zeigen, dass die Bromeliaceae vor rund 100 Millionen Jahren (My) am Guayana-Schild entstanden sind und sich radial in die angrenzenden Gebiete vor ca. 16-13 My ausgebreitet haben. Heute noch lebende Entwicklungslinien entstanden vor 20-5 My. Die Andenhebung begünstigte die Artbildung innerhalb der Kern-Tillandsioideae vor etwa 14,2 My und die der Bromelioideae vor 10,1 My, wobei letztere ihre größte Vielfalt am brasilianischen Schild aufgrund der Ausbreitung von den Anden her hat. Die heute artenreichsten Gattungen mit einer

erhöhten Artbildungsrate vor 5-4 Ma sind nicht vor 8,7 My erschienen, was höchstwahrscheinlich der Grund für die vergleichsweise niedrige Sequenzdivergenz ist.

Um die Verwendbarkeit von nukleären Regionen für phylogenetische Untersuchungen in den Bromelioideae zu testen, wurden DNS-Sequenzen von einem Abschnitt des in geringer Kopienzahl vorliegenden nukleären Gens Phosphoribulokinase (*PRK*) und fünf Plastiden-Marker (*matK*, 3'*trnK* Intron, *trnL* Intron, *trnL-trnF* und *atpB-rbcL*) untersucht. Stammbäume aus Analysen der *PRK*-Daten widersprechen nicht den Ergebnissen, die mit Hilfe von Plastiden-Markern erhalten werden. Die *PRK*-Matrix zeigt eine deutlich höhere Anzahl an potenziellen PICs (phylogenetisch informative Merkmale), die die Auflösung und die Absicherung der resultierenden Stammbäume verbessert, als der Plastiden-Datensatz (16,9% vs. 3,1%). Obwohl *PRK* nicht in der Lage ist die Verwandtschaftsbeziehungen vollständig aufzulösen, liefert die kombinierte Analyse mit den Plastiden-Markern eine gute Unterstützung für mehrere zuvor unsichere Verwandtschaftsverhältnisse. Die früh abzweigenden Entwicklungslinien konnten identifiziert werden („basal bromelioids“) und der Rest der Unterfamilie ist in einem hoch unterstützten Ast vereinigt („eu-bromelioids“). Die Ergebnisse zeigen, dass die taxonomischen Beziehungen von Gattungen innerhalb der „core bromelioids“ verflochten und schwierig zu lösen sind. Mehrere Gattungen erscheinen polyphyletisch und *Aechmea* sowie *Quesnelia* bleiben die komplexesten Gattungen der Unterfamilie. Most-Parsimonious-Rekonstruktionen von Merkmalszuständen für Trichterhabitus und Kelchblattsymmetrie zeigen, dass beide Merkmale innerhalb der Unterfamilie nur wenigen morphologischen Veränderungen unterworfen waren und daher nicht so stark homoplastisch sind wie bisher angenommen.

Die vergleichende Studie von nukleären DNS-Sequenzen innerhalb der Tillandsioideae zeigt, dass einige nukleäre Marker in der Lage sind, mehr Informationen und eine höhere Auflösung in den Stammbäumen zu erzielen als Plastiden-Marker. Allerdings ist ihre Verwertbarkeit nicht nur von der Sequenzvariabilität, sondern auch von methodischen Herausforderungen der traditionellen Sanger-Sequenzierung abhängig. Der „internal transcribed spacer“ der nukleären ribosomalen DNS (ITS nrDNS) ist aufgrund von vorhandenen starken Sekundärstrukturen, die Probleme bei der Amplifikation und Sequenzierung bereiten, sowie seiner geringen Anzahl an PICs kein geeigneter Marker zum Auflösen tieferer Knoten in phylogenetischen Untersuchungen bei den Bromeliaceae. Amplifizierte Fragmente der Gene Malat-Synthase (*MS*) und RNA-Polymerase-II, Beta-Untereinheit (*RPB2*) sind aufgrund ihrer geringen Länge und der begrenzten Anzahl an PICs nicht sonderlich hilfreich. Glucose-6-Phosphat-Isomerase (*PGIC*), Nitrat-Reduktase (*NIA*) und Xanthin-Dehydrogenase (*XDH*) müssen weiter untersucht werden. Phosphoribulokinase (*PRK*) und Phytochrom C (*PHYC*) sind nützliche nukleäre Marker und bringen eine gute Auflösung innerhalb vieler Bereiche der Stammbäumen, jedoch sind einige Verwandtschaftsverhältnisse nicht aufgelöst oder nur schlecht unterstützt.

Die kombinierte Analyse der Kern-DNS-Sequenzdaten (*PRK*, *PHYC*) mit den bereits vorhandenen Plastiden-DNS-Sequenzdaten (*atpB-rbcL*, *matK*, *rbcL*, einem Teilstück des *rbcL-accD*, *rps16* intron, einem Teilstück des *trnK* intron, *trnL* intron und *trnL-trnF*) zeigt eine deutliche Steigerung der Auflösung innerhalb der Stammbäume der Tillandsioideae. Basierend auf morphologischen Merkmalen konnten neun bisher akzeptierte Gattungen neu definiert und drei Gattungen neu beschrieben werden. Für morphologisch abweichende Artengruppen innerhalb von *Racinaea* und *Tillandsia* werden zwei neue Untergattungen aufgestellt. *Viridantha* wird auf Untergattungsniveau herabgestuft. Geringe Probenahme im *Cipuropsis-Mezobromelia*-Ast und fehlende Unterstützung für Verwandtschaftsgruppen innerhalb von *Tillandsia* verhindern eine taxonomische Beschreibung weiterer Artengruppen.

Introduction

Molecular markers for plant phylogenetics

Since the first extensive publication of an angiosperm phylogeny based on plastid *rbcL* sequence data (Chase & al., 1993), molecular studies in plants using DNA sequences have become popular. Advancements in sequencing technologies in both hardware and software have facilitated the addition of vast amounts of sequence information to public databases (e.g., GenBank) and a great number of published phylogenetic studies over the last 20 years. Milestones in sequencing technology have included the development of the chain-termination method (Sanger-sequencing) by Sanger & al. (1977) and the release of affordable automated plate (ABI PRISM 377 Genetic Analyzer, Life Technologies) and, later, capillary sequencers (e.g., Applied Biosystems 3730 DNA analyser, Life Technologies; Amersham Bioscience MegaBACE 4000, GE Healthcare) for detection of fluorescently labelled fragments. Preparation, handling, and throughput of sequences improved greatly, and generating sequence data became a routine exercise. Since 2005, next generation sequencing (NGS) technologies have enabled high-throughput sequencing using fluorescence (454, Roche; Solexa, Illumina; SOLiD, Life Technologies) or pH change measurements (Ion Torrent, Life Technologies) for detection of incorporated nucleotides. Although different applications have recently been developed, high costs, handling and analysing the huge amounts of sequence data, and the rather uncontrolled sequencing of genomic fragments are still limiting factors for general application of NGS methodologies to plant phylogenetics.

During the period 1993–2000 the most important sources of data for reconstructing plant phylogenies were a small number of plastid DNA loci and the multi-copy 18S-5.8S-26S nuclear ribosomal DNA (nrDNA) repeat unit, e.g.,

(1) plastid DNA markers: e.g., *atpB* (e.g., Hoot & al., 1995); *atpB-rbcL* (e.g., Manen & al., 1994); *matK* (e.g., Johnson & Solitis, 1994, 1995; Liang & Hilu, 1996); *ndhF* (e.g., Olmstead & Reeves, 1995; Olmstead & al., 2000); *psbA-trnH* (e.g., Sang & al., 1997); *rbcL* (e.g., Nickrent & Soltis, 1995); *rps16* intron (e.g., Oxelman & al., 1997); *trnT-trnL-trnF* (e.g., Taberlet & al., 1991; Gielly & Taberlet, 1996; Sang & al., 1997);

(2) nuclear ribosomal DNA markers: e.g., 18S nrDNA (e.g., Nickrent & Soltis, 1995); 26S nrDNA (e.g., Bult & al., 1995; Kuzoff & al., 1998); external transcribed spacer (ETS nrDNA) (e.g., Baldwin & Markos, 1999); internal transcribed spacer (ITS nrDNA) (e.g., White & al., 1990; Baldwin, 1992; Sun & al., 1994; Baldwin & al., 1995; Sang & al., 1995; Blattner, 1999; Douzery & al., 1999).

More conserved gene regions (*atpB*, *rbcL*, 18S nrDNA, 26S nrDNA) were applied at higher taxonomic levels, faster evolving genes (*matK, ndhF*) at intermediate levels, and more variable introns and spacer regions (*atpB-rbcL*, *psbA-trnH*, *rps16* intron, *trnT-trnL-trnF*, ETS nrDNA, ITS nrDNA) at interspecific levels. Although new primers for novel markers had been published (e.g., Demesure & al., 1995; Dumolin-Lapegue & al., 1997; Small & al., 1998), most studies

continued to use these frequently sequenced plastid loci. Comparative studies on several plastid DNA markers including information on existing or new universal primers (Shaw & al., 2005, 2007) have increased the number of applicable plastid DNA markers and primers, but also promoted the further use of plastid DNA sequences solely. Additional published nrDNA primers (e.g., Gruenstaeudl & al, 2009) continue to facilitate the usage of the ITS nrDNA (e.g., Russell & al., 2010a; Hřibová & al., 2011).

The first low-copy nuclear DNA genes investigated for their phylogenetic utility belonged to the phytochrome gene family (*PHYA*, *PHYB*, *PHYC*, *PHYD*, *PHYE*; e.g., Mathews & al., 1995; Mathews & Sharrock, 1996, 1997; Mathews & Donoghue, 1999; Mathews & al., 2000). Only a few additional studies had utilized other low-copy nuclear DNA loci (e.g., *ADH*: Morton & al., 1996; Sang & al., 1997; Sang & Zhang, 1999; *ncpGS*: Emshwiller & Doyle, 1999; *NIA*: Howarth & Baum, 2002; *PEPC*: Gehrig & al., 1998, 2001; *RPB2*: Denton & al., 1998). It has been shown that low-copy nuclear DNA sequences are more variable and phylogenetically informative markers than plastid DNA regions (e.g., Strand & al., 1997; Sang, 2002; Mort & Crawford, 2004; Small & al., 2004), but direct sequencing is unfeasible in many cases because of their biparental inheritance and high a frequency of heterozygous individuals carrying alleles of different lengths. In the case of multi-copy genes, differentiating between paralogous and orthologous sequences becomes problematic. The need for cloning, and difficulties in interpreting results due to evolutionary processes like hybridization (e.g., Sang, 2004; Ma & al., 2010) or incomplete lineage sorting (e.g., Piñeiro & al., 2009; Willyard & al., 2009) have limited their usage. However, low-copy nuclear DNA sequences have been employed with success for some plant groups, e.g., angiosperms (*RPB2*: Oxelman & al., 2004; *XDH*: Morton & al., 2011), Arecaceae (*MS*: Lewis & Doyle, 2001, 2002; Thomas & al., 2006; *PRK*: Lewis & Doyle, 2002; *RPB2*: Thomas & al., 2006), Asteraceae (*PGIC*: Ford & al., 2006; Stuessy & al., 2011; *GADPH*: Vaezi & Brouillet, 2009), Orchidaceae (*XDH*: Górniak & al., 2010), Paeoniaceae (*G3PAT*: Tank & Sang, 2001), and Passifloraceae (*ncpGS*: Yockteng & Nadot, 2004; Clarkson & al., 2010). Nevertheless, most phylogenetic studies in flowering plants are still based on maternally inherited, single-copy plastid DNA regions and the ITS nrDNA, which is usually well homogenized due to the phenomenon of concerted evolution. The absence of universal primers for low-copy nuclear genes has limited their amplification in a wider range of taxonomic levels in angiosperms.

In certain plant groups the study of few plastid DNA markers together with the ITS nrDNA has been shown to provide insufficient information for reconstructing phylogenies at lower taxonomic levels (e.g., Barfuss & al., 2005; Lukas, 2010; Safer, 2011). Two hypotheses can be formulated, either that in certain plant groups the plastid genome and nrDNA regions evolve at a much slower rate (e.g., Gaut & al., 1992, 1997), or that extant species have undergone a rapid diversification process in their recent history (e.g., Cronn & al., 2002; Hughes & Eastwood, 2006), which facilitated great morphological diversity, but comparatively little sequence divergence. In both cases a combined analysis of several plastid DNA markers (e.g., Chase & al., 2006; Russell & al., 2010b) or the study of faster-evolving low-copy nuclear loci (e.g., Álvarez & al., 2008; Russell & al., 2010a) are possible solutions.

Characteristics, taxonomic history, and previous molecular studies in Bromeliaceae

Bromeliaceae is among the most conspicuous and species-rich groups of monocot angiosperms, constituting an early-diverging lineage within the order Poales (Givnish & al., 2007, 2010, see Figure 1; APG III, 2009). They are distributed in various, ecologically diverse habitats in tropical and subtropical regions of the New World ranging from the southern United States

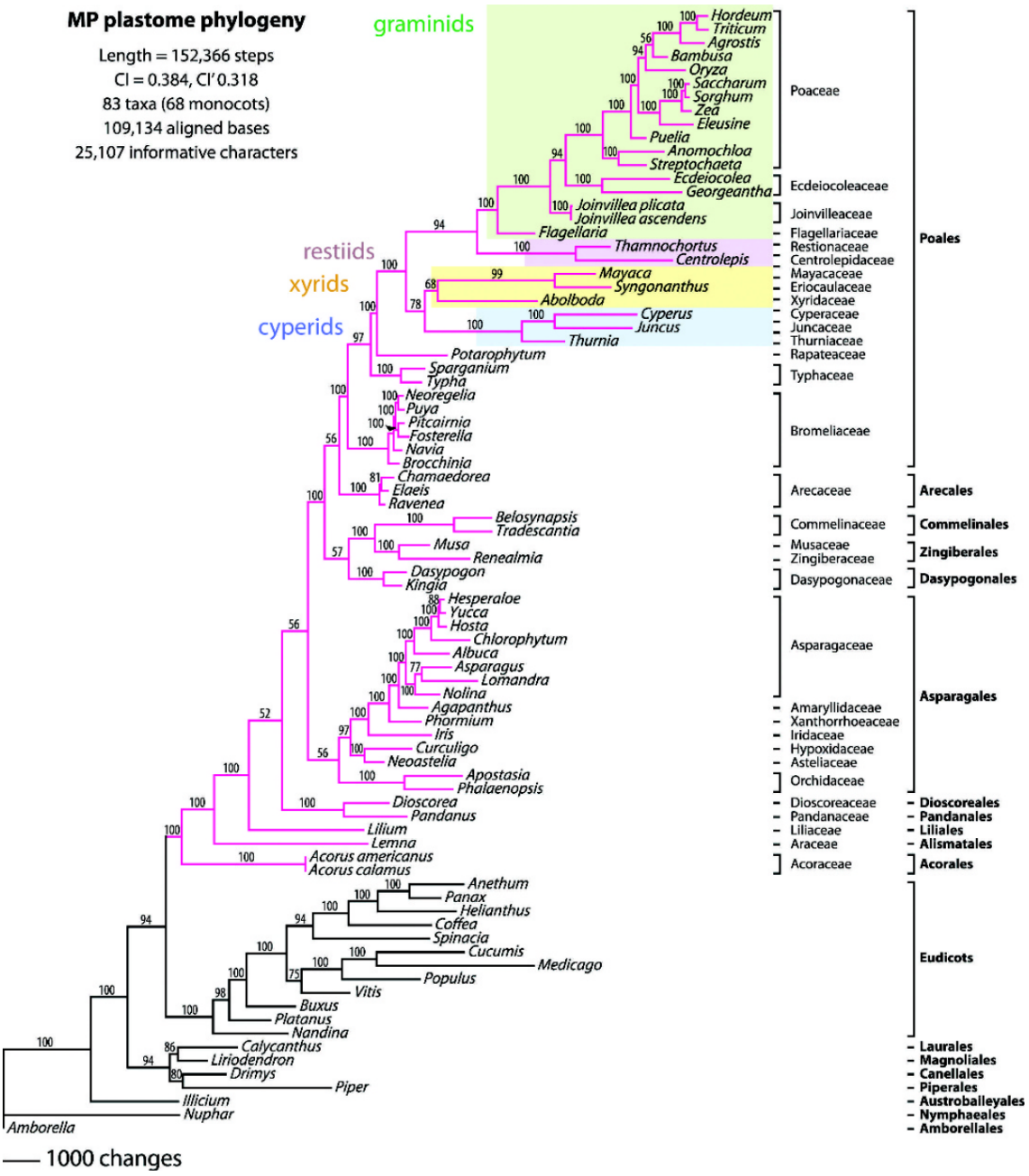

Figure 1. Phylogram of the single most parsimonious tree resulting from analysis of the plastome data showing the phylogenetic position of Bromeliaceae as the earliest-diverging lineage of Poales. Branch lengths are proportional to the number of inferred substitutions along each branch. Bootstrap support for each node is shown above the corresponding branch. Monocots are highlighted with magenta branches; cyperids, xyrids, restiids, and graminids, with colored boxes. **(Taken from Givnish & al., 2010).**

(Virginia) to Patagonia in southern Argentina (Smith & Downs, 1974; Givnish & al., 1997; Benzing 2000). Their greatest species diversity is found in mountainous regions at elevations between 1000 and 2500 m.a.s.l. throughout the entire distributional range. Only one species, *Pitcairnia feliciana*, is found outside this area in Western Africa, which has been shown to be a result of long-distance dispersal about 10 Ma (Givnish & al., 2007). This supports the hypothesis that extant bromeliads have developed much later than the Gondwanan continent breakup, similarly to other flowering plant families (e.g., Cactaceae).

Bromeliads are morphologically very distinctive, hence their inclusion in a separate order Bromeliales. However, a relationship to Velloziaceae has been proposed by cladistic analyses of morphological characters (Gilmartin & Brown, 1987) and the early molecular studies supported genetic affinities to Rapateaceae or Mayacaceae (Clark & al., 1993; Duvall & al., 1993). The family is characterized by typical monocot flowers (K_3, C_3, A_{3+3}, $G_{(3)}$) arranged in often showy inflorescences, distinctive leaf rosettes that are often water-impounding ("tanks"), and leaves that bear absorptive trichomes for water and nutrient uptake. The latter two features have provided key innovations to favour colonization of habitats of alternating water and nutrient supply as epiphytes (e.g., Benzing, 2000; Crayn & al., 2004, see Figure 2). Additional adaptions to drought stress, like succulence and the evolution of the CAM photosynthetic pathway, have also enabled their establishment in less competitive, extreme and rocky environments as lithophytes (e.g., McWilliams, 1974; Givnish & al., 2007). Several species of the family are of important horticultural value and many botanical gardens, amateur botanists, and private collectors host a large number of species *ex situ* in living plant collections.

In Bromeliaceae, ca. 3,140 species are classified into 58 genera, which have traditionally been assigned to three subfamilies (Smith & Downs, 1974, 1977, 1979; Smith & Till, 1998):

(1) Bromelioideae: inferior ovarys, baccate fruits, and serrate or rarely entire leave margins;
(2) Pitcairnioideae: superior to partly inferior ovarys, capsular fruits, bicaudate, winged or rarely naked seeds, and serrate or rarely entire leave margins;
(3) Tillandsioideae: superior to semi- inferior ovarys, capsular fruits, plumose seed appendages, and exclusively entire leave margins.

Recent molecular studies have revealed that both Bromelioideae and Tillandsioideae are monophyletic and well characterized by their traditional morphology (e.g., Terry & al., 1997a, b; Crayn & al., 2004; Barfuss & al., 2005; Givnish & al., 2004, 2007; Schulte & al., 2005). Pitcairnioideae is paraphyletic and morphological characters used to define this subfamily are plesiomorphic. Therefore Givnish & al. (2007, see Figure 3) established five additional subfamilies based on DNA sequence data and a re-evaluation of morphological characters to circumscribe natural, monophyletic lineages: Brocchinioideae, Lindmanioideae, Hechtioideae, Navioideae, and Puyoideae.

The most species-rich and taxonomically complicated subfamilies of Bromeliaceae are Bromelioideae and Tillandsioideae. Subfamily Bromelioideae is the second largest with 32 genera and ca. 800 species (Smith & Till, 1998; Luther 2010). Although distributed over nearly the whole range of the family, the centre of diversity is found in the Atlantic rain forest of south-eastern Brazil (Smith & Downs, 1979). The largest and taxonomically most complex genus of Bromelioideae is *Aechmea* (260 spp.). Subfamily Tillandsioideae is the largest subfamily with nine generally accepted genera and ca. 1,300 species (Smith & Downs, 1977; Smith & Till, 1998; Luther, 2010; Luther & Rabinowitz, 2010) found throughout the distribution range of Bromeliaceae, indicating the greatest potential to adapt to extreme environments. Centres of diversity are the northern Andes in Peru, Ecuador and Colombia, the Atlantic rain forest of south-eastern Brazil, and Central America (Smith & Downs, 1977). It is considered the most morphologically diverse subfamily, hosting the three large genera *Guzmania* (210 spp.), *Vriesea* (266 spp.), and the most species-rich genus of Bromeliaceae, *Tillandsia* (626 spp.).

Generic and subgeneric delimitations within subfamilies based on morphology have been shown to be problematic and in need of urgent revision (e.g., Smith & Kress, 1989, 1990;

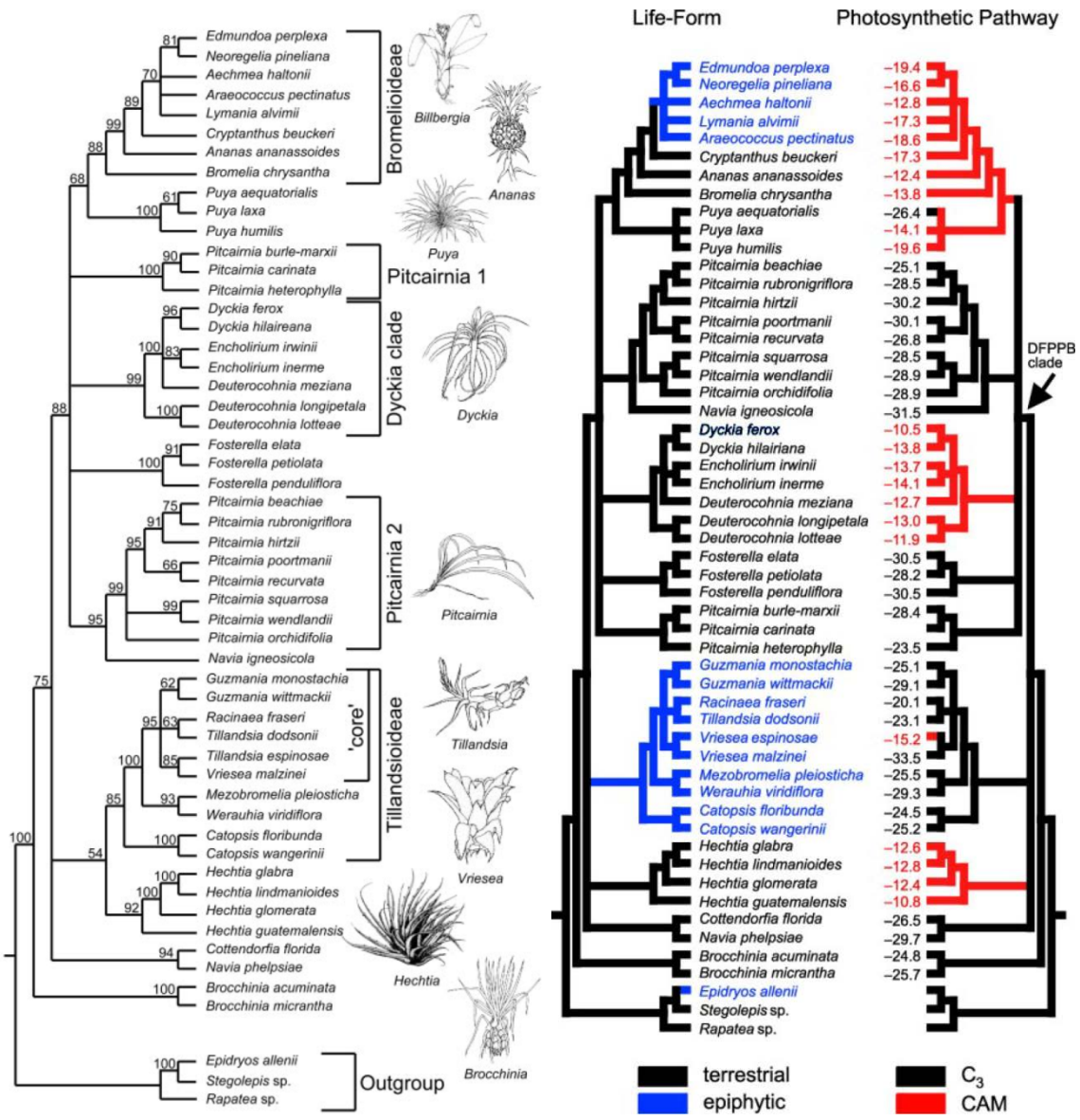

Figure 2. Strict consensus tree and most-parsimonious reconstructions of the combined *matK* and *rps16* intron data for 51 species of Bromeliaceae showing the evolution of life-form and photosynthetic pathway. Bootstrap values are indicated above the corresponding branches. Carbon-isotope ratios ($\delta^{13}C$ values in ‰) are shown for the taxa analysed. The derived character-states "epiphytic" and "CAM" are highlighted in blue and red, respectively. **(Taken from Crayn & al., 2004).**

Brown & Terry, 1992; Grant, 1993b, 1995a; Read & Baensch, 1994; Betancur & Miranda-Esquivel, 1999; Brown & Leme, 2005; Betancur & Salinas, 2006; de Sousa & Wendt, 2008). Several studies investigating distinctive morphological features, which were not yet applied to the taxonomy, have provided evidence that many characters are plesiomorphic or homoplastic and hence in their current application fail to delimit natural groups within Bromeliaceae. Inter- and infrageneric relationships, especially of Bromelioideae and Tillandsioideae, still remained poorly understood and taxonomic circumscriptions inadequate, because studies have relied on a very restricted number of species in comparison to the species richness of the subfamilies and, often, morphological characters are missing from herbarium specimens. Repeated changes of taxonomic boundaries by transferring or excluding species, changes in taxonomic ranks, and newly proposed segregates since the last comprehensive monograph of the family (Smith

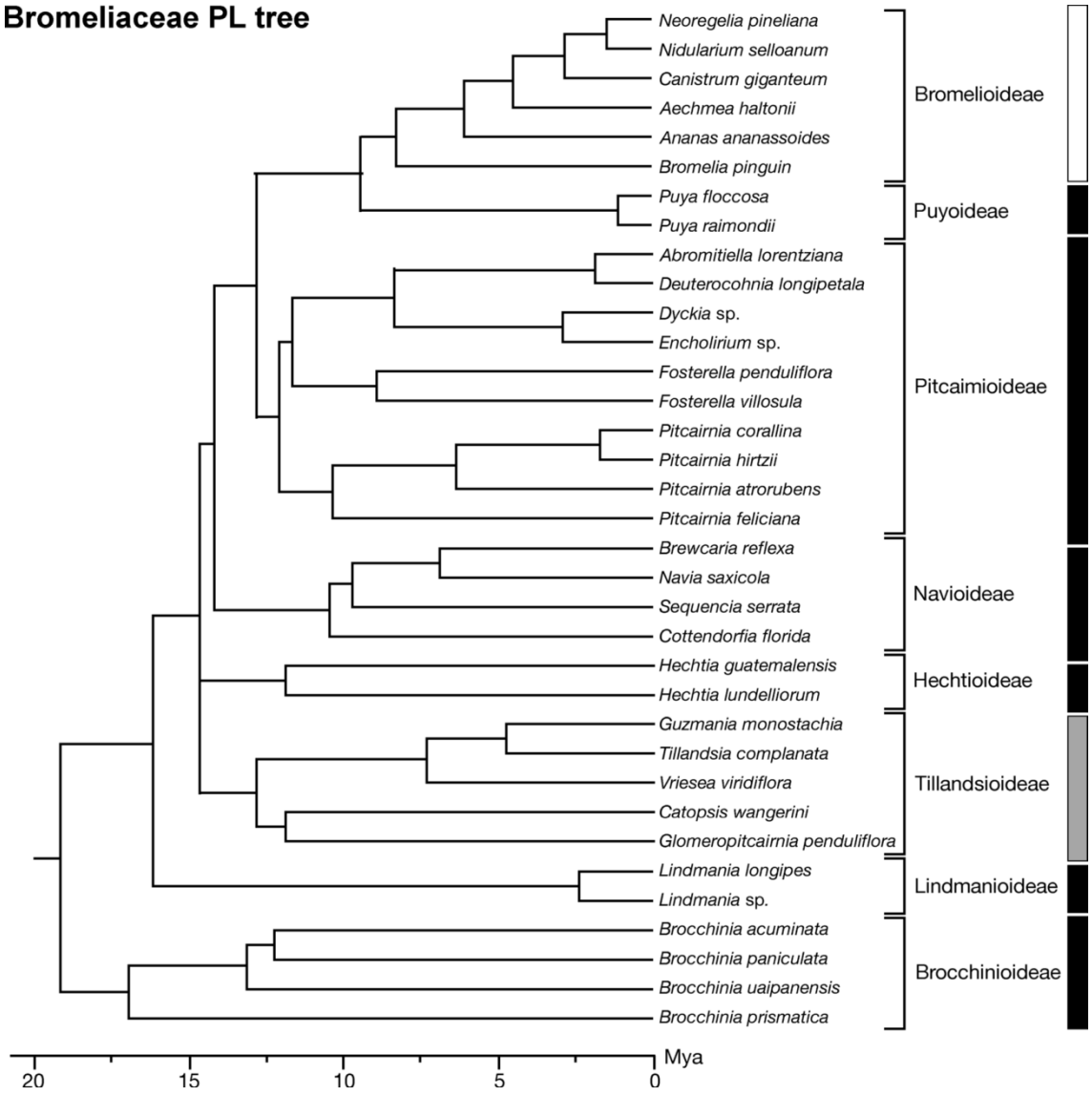

Figure 3. Ultrametric tree for Bromeliaceae based on cross-verified penalized likelihood, showing inferred chronology of cladogenesis over the past 20 My. The new subfamilial classification is highlighted by brackets; membership of subfamilies in the three traditional subfamilies is indicated by shaded bars. Hollow bar = Bromelioideae; gray bar = Tillandsioideae; solid bars = Pitcairnioideae. **(Taken from Givnish & al., 2007).**

& Downs, 1974, 1977, 1979), as well as the greatly increased number of newly described species (Luther, 2010) indicate an urgent need for modern taxonomic revision based on reliable morphological characters.

The first molecular studies on Bromeliaceae used restriction site variation in plastid DNA (Ranker & al., 1990) to infer phylogenetic relationships within bromeliads. Early studies that included sequence data used the *rbcL* gene to clarify either subfamilial relationships or familial, ordinal and superordinal relationships of Bromeliaceae and related monocot families (Clark & al., 1993). Their results confirmed a monophyletic origin of Bromeliaceae, but relationships of the three subfamilies could not be clearly resolved, due to limited taxon sampling. Two studies on the *ndhF* gene from the small single-copy region of the plastid genome were published by Terry & al. (1997a, b). These studies focused on subfamilial relationships (1997a, 30 bromeliad species) and relationships below the subfamilial level of Tillandsioideae (1997b, 25 tillandsioid species). Since the resolution of trees obtained from analyses of *ndhF* sequences was rather

low, subsequent publications used the more rapidly evolving *trnL* intron (Horres & al., 2000; 64 bromeliad species), *matK,* and *rps16* intron sequences (Crayn & al., 2000, 2004; 51 bromeliad species) to construct phylogenies and infer evolution of certain traits of bromeliads (e.g., CAM photosynthesis, epiphytic habit). However, these results also showed low sequence divergence among taxa and it was initially speculated that the plastid genome of Bromeliaceae evolves at a much slower rate than in other angiosperm lineages (Gaut & al., 1992, 1997; Givnish & al., 2004). The greatest drawbacks of early bromeliad phylogenetic publications were their reliance on only a few sequences of the plastid genome and the inclusion of a very limited set of species. Therefore subsequent studies focused on the large subfamilies Tillandsioideae (Barfuss & al., 2005) and Bromelioideae (Schulte & Zizka, 2008) with a greater selection of taxa and four to seven plastid markers in concatenated data matrices for analyses. Although it was possible to resolve major phylogenetic units within the family and subfamilies, the results still showed low resolution and low support for some deeper nodes. Sequence divergence at generic and interspecific levels was still comparatively low.

Nuclear DNA markers were only recently included in phylogenetic studies of bromeliads. Studies using limited sets of taxa were undertaken (1) for Bromelioideae using partial sequences of the gene phosphoribulokinase (*PRK*) (Schulte & al., 2009; see chapter 2); (2) for *Tillandsia* subg. *Tillandsia* using sequences of the internal transcribed spacer 2 (ITS2) and the ETS nrDNA (Chew & al., 2010); (3) for *Aechmea* sequencing parts of the genes RNA polymerase II, beta subunit (*RPB2*), glyceraldehyde-3-phosphate dehydrogenase (*G3PDH*), and ETS nrDNA (Sass & Specht, 2010); (4) for *Puya* using parts of exon 1 of phytochrome C (*PHYC*) (Jabaily & Sytsma, 2010); and (5) for *Alcantarea* investigating *FLORICAULA*/*LEAFY* (*FLO*/*LFY*) (Versieux & al., 2012). Despite the increased usage of nuclear regions, relationships within Bromelioideae and Tillandsioideae still remain inadequately resolved, particularly within complicated and species-rich genera such as *Aechmea*, *Tillandsia*, and *Vriesea*.

Taxonomic history and previous molecular studies in Tillandsioideae

In Tillandsioideae more than 1,300 species are classified into nine accepted genera: Alcantarea (28 spp.), *Catopsis* (18 spp.), *Glomeropitcairnia* (2 spp.), *Guzmania* (210 spp.), *Mezobromelia* (9 spp.), *Racinaea* (65 spp.), *Tillandsia* (626 spp.), *Vriesea* (266 spp.), and *Werauhia* (87 spp.). The large genera *Tillandsia* and *Vriesea* as well as *Werauhia* are further divided into subgenera or sections (Till, 2000a, b). Since the last monograph for Tillandsioideae by Smith & Downs (1977) there have been changes in taxonomic limits (*Tillandsia*, *Vriesea*), establishment of new genera (*Alcantarea*: Grant, 1995; *Werauhia*: Grant, 1995; *Racinaea*: Spencer & Smith, 1993; *Viridantha*: Espejo-Serna, 2002), and transfers of species to other genera (e.g., xerophytic *Vriesea* species: Grant, 1993b, 2005). These changes have demonstrated the unreliability of previous taxonomies based on traditional morphological characters (e.g., flower arrangement, corolla tube connations, stamen and style position, presence vs. absence of petal appendages). Additional studies on gross morphology of flowers have supported this view (e.g., Brown & Gilmartin, 1984, 1989; Böhme, 1988; Gross, 1988; Gortan, 1991; Halbritter, 1992) and shown that the three largest genera (*Guzmania*, *Tillandsia*, *Vriesea*) and *Mezobromelia* are paraphyletic. The new description and resurrection, respectively, of the genera *Viridantha* (Espejo-Serna, 2002) and *Sodiroa* (Betancur & Miranda-Esquivel, 1999) are not generally accepted (e.g., Barfuss & al., 2005; Luther 2010). The remaining five currently accepted genera seem to be well circumscribed, with *Catopsis* and *Glomeropitcairnia* being the most distinctive genera of the subfamily (Smith & Till, 1998; Till, 2000a).

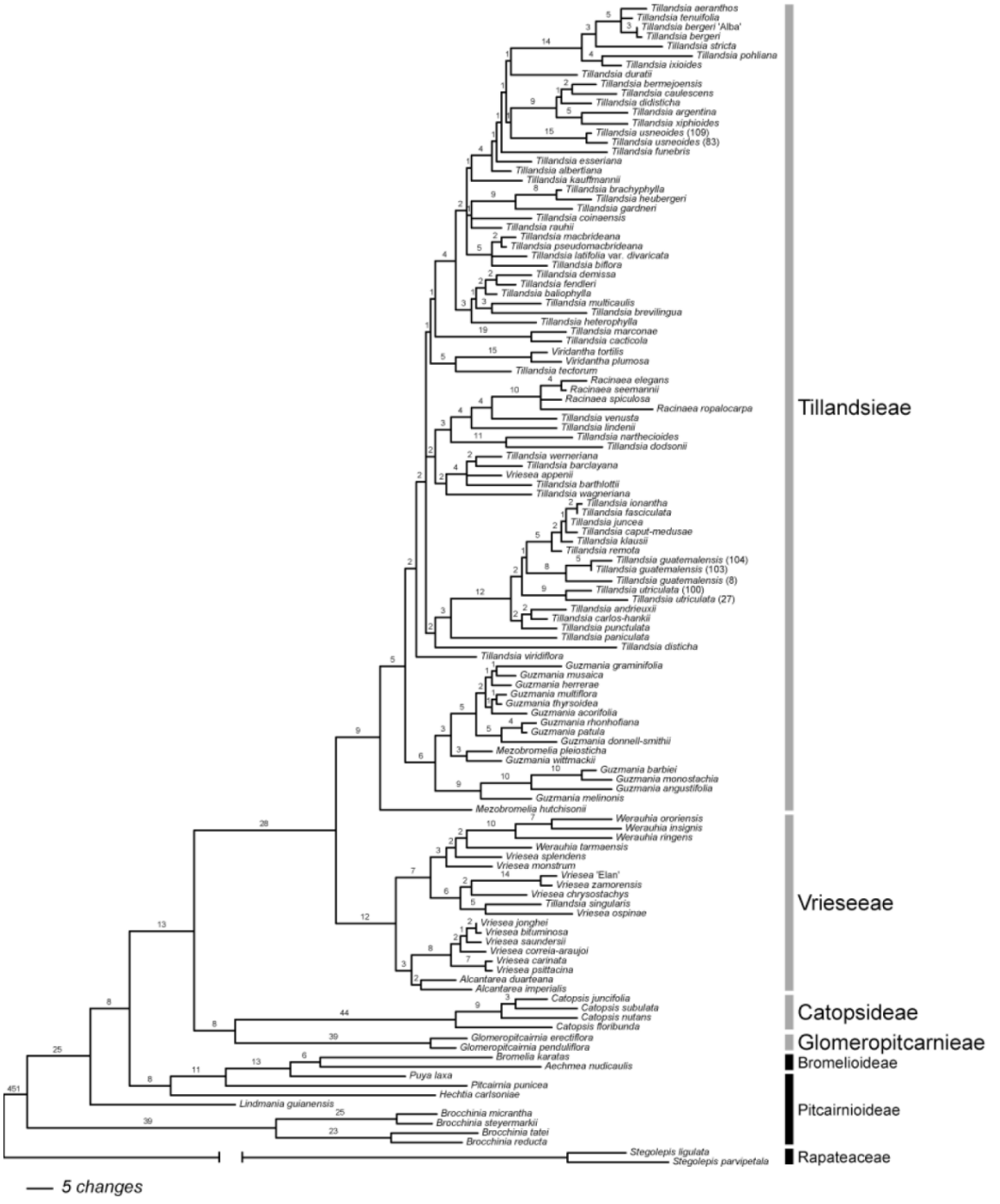

Figure 4. Phylogram of one most parsimonious tree of 110 Tillandsioideae and 12 outgroup taxa obtained from combined analysis of seven plastid markers. Branch length is given above the branches. Gray bars indicate the tribal classification of Tillandsioideae, black ones the other subfamilies and the super-outgroup (Rapateaceae). **(Taken from Barfuss & al., 2005).**

The two earliest molecular studies focusing on Tillandsioideae were restricted to a single marker and a limited sample set (*ndhF*: Terry & al., 1997b; *trnL* intron: Horres & al., 2000). The outcomes were correspondingly poor: although they supported the monophyly of Tillandsioideae and the distinct positions of *Catopsis* and *Glomeropitcairnia*, relationships of the remaining genera were mostly unresolved. A more recent study with an increased taxon sampling and seven plastid DNA markers (Barfuss & al., 2005; see Figure 4) confirmed these results but was

also able to resolve major taxonomic units and clarify relationships of additional genera (*Alcantarea*, *Guzmania*, *Werauhia*). The remaining genera turned out to be paraphyletic (*Vriesea*, *Tillandsia*) and/or poorly genetically differentiated (*Racinaea*, *Tillandsia*) as evident from short and weakly- or unsupported branches. This demonstrated the need for more rapidly evolving markers, preferably from the nuclear genome. Two recently published studies utilized either nrDNA regions (ITS2 and ETS nrDNA for *Tillandsia* subg. *Tillandsia*: Chew & al., 2010) or a single low-copy nuclear gene (*FLO*/*LFY* for *Alcantarea*: *Versieux* & al., 2012), but were restricted to small subgroups of Tillandsioideae and a poor sample selection.

Aims of the present study

The scope of the present investigation was defined by three main questions:

(4) Do additional sequence data from the plastid genome and a wider sampling within Bromeliaceae provide a better resolved, robust phylogenetic framework? What are the reasons for the low DNA sequence divergence observed up to now?
(5) Can nuclear DNA sequences be successfully implemented for phylogenetic reconstruction? What are the challenges to optimize nuclear markers, and do they perform better than plastid loci?
(6) Can the resulting phylogenies based on plastid and nuclear DNA sequences together with the re-evaluated morphological characters provide a reasonable, stable classification?

To answer these questions the work was divided into three parts, which correspond to the three chapters of the present thesis: (1) Application of additional plastid DNA sequence data to phylogenetic reconstruction in Bromeliaceae to test the eight-subfamily classification and to strengthen hypotheses concerning biogeography, origin, age, and key trait evolution of extant bromeliad lineages, pp. 27–54; (2) Optimization of nuclear DNA markers and their application to phylogenetic reconstructions within Bromeliaceae (in comparison to plastid DNA markers) to answer questions of relationships and character evolution within Bromelioideae and Tillandsioideae, pp. 55–116; (3) Implementation of phylogenetic results in the classification and taxonomy of Tillandsioideae, pp. 117–206.

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Part 1

Application of plastid DNA markers in phylogenetic reconstructions of Bromeliaceae

Chapter 1

Phylogeny, adaptive radiation, and historical biogeography in Bromeliaceae: Insights from an eight-locus plastid phylogeny

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Status: published, *American Journal of Botany* 98 (5): 872–895. 2011.

Contribution: data collection, preliminary data analyses, manuscript writing/editing.

American Journal of Botany

American Journal of Botany 98(5): 872–895. 2011.

Phylogeny, adaptive radiation, and historical biogeography in Bromeliaceae: Insights from an eight-locus plastid phylogeny[1]

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- *Premise*: Bromeliaceae form a large, ecologically diverse family of angiosperms native to the New World. We use a bromeliad phylogeny based on eight plastid regions to analyze relationships within the family, test a new, eight-subfamily classification, infer the chronology of bromeliad evolution and invasion of different regions, and provide the basis for future analyses of trait evolution and rates of diversification.
- *Methods*: We employed maximum-parsimony, maximum-likelihood, and Bayesian approaches to analyze 9341 aligned bases for four outgroups and 90 bromeliad species representing 46 of 58 described genera. We calibrate the resulting phylogeny against time using penalized likelihood applied to a monocot-wide tree based on plastid *ndhF* sequences and use it to analyze patterns of geographic spread using parsimony, Bayesian inference, and the program S-DIVA.
- *Results*: Bromeliad subfamilies are related to each other as follows: (Brocchinioideae, (Lindmanioideae, (Tillandsioideae, (Hechtioideae, (Navioideae, (Pitcairnioideae, (Puyoideae, Bromelioideae)))))). Bromeliads arose in the Guayana Shield ca. 100 million years ago (Ma), spread centrifugally in the New World beginning ca. 16–13 Ma, and dispersed to West Africa ca. 9.3 Ma. Modern lineages began to diverge from each other roughly 19 Ma.
- *Conclusions*: Nearly two-thirds of extant bromeliads belong to two large radiations: the core tillandsioids, originating in the Andes ca. 14.2 Ma, and the Brazilian Shield bromelioids, originating in the Serro do Mar and adjacent regions ca. 9.1 Ma.

Key words: Andes; Bromeliaceae; bromeliads; epiphytes; Guayana Shield; historical biogeography; neotropics; Poales; Serra do Mar; tank formation.

[1]Manuscript received 13 February 2010; revision accepted 9 February 2011.

The authors gratefully acknowledge financial support for this investigation by grants from the National Science Foundation to P.E.B., K.J.S., and T.J.G. (DEB-9981587), T.J.G. (DEB-0830036), K.J.S. (DEB-0431258), and G.L.B. and T.M.E. (DEB-0129446 and DEB-0129414), and from the Hertel Gift Fund to T.J.G., from the Commission for Interdisciplinary Ecological Studies (KIÖS) at the Austrian Academy of Sciences (ÖAW, 2007-02 to W.T. and M.H.J.B., and from the Deutsche Forschungsgemeinshaft (ZI 557/7-1, SCHU 2426/1-1) and the Hessian initiative for the development of scientific and economic excellence (LOEWE) at the Biodiversity and Climate Research Centre, Frankfurt am Mein, to G.Z. and K.S. Plant material was kindly supplied by the Selby Botanical Garden, the Palmengarten Frankfurt am Main, and the Botanical Gardens of Heidelberg. Alejandra Gandalfo provided some useful references on the geological history of the Andes. Many thanks to two anonymous reviewers and D. E. Soltis for several helpful suggestions.

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doi:10.3732/ajb.1000059

The family Bromeliaceae (58 genera, ca. 3140 species) constitute one of the most morphologically distinctive, ecologically diverse, and species-rich clades of flowering plants native to the tropics and subtropics of the New World (Fig. 1). Bromeliads range from mist-shrouded tepuis in Venezuela to sun-baked granitic outcrops of the Brazilian Shield, from cloud forests in Central and South America to the cypress swamps of the southern United States, and from the frigid Andean puna to the arid Atacama (Smith and Downs, 1974; Givnish et al., 1997; Benzing 2000). Their distinctive leaf rosettes often impound rainwater in central tanks, possess the CAM photosynthetic pathway, and bear absorptive trichomes, providing mechanisms to weather drought and obtain or conserve nutrients on rocks and exposed epiphytic perches (Pittendrigh, 1948; McWilliams, 1974; Crayn et al., 2004; Givnish et al., 2007; Schulte et al., 2009). Bromeliad tanks also house a great diversity of insects—including some with substantial impact on human health—and other arthropods, as well as crabs, frogs, salamanders, and snakes.

In a hectare of cloud forest, these tanks can sequester tens of thousands of liters of rainwater and trap hundreds of kilograms of humus high in the canopy and provide key food sources for many primates and birds (Paoletti et al., 1991; Leme, 1993; Sillett, 1994; Richardson, 1999; Benzing, 2000; Acevedo et al., 2008). Some tank bromeliads are directly carnivorous (Fish, 1976; Frank and O'Meara, 1984; Givnish et al., 1984, 1997), and at least one is known to benefit from the prey captured by inquiline spiders (Romero et al., 2006). Many tank bromeliads are protected and/or fed by ants (Benzing, 1970, 2000; McWilliams, 1974; Givnish et al., 1997). Pollinators include a wide variety of insects, as well as hummingbirds, bats, and a few perching birds (Benzing, 1980, 2000; Luther, 1993; Beaman and Judd, 1996; Smith and Till, 1998; Buzato et al., 2000; Krömer et al., 2006; Tschapka and von Helversen, 2007). The inflorescences of *Puya raimondii* are the most massive of any flowering plant, while those of some dwarf *Brocchinia* and *Tillandsia* are only a few centimeters in height (Fig. 1). Finally, bromeliads contribute a large share of the total species richness of vascular epiphytes in neotropical forests, are particularly diverse at midelevations, and exhibit increasingly narrow endemism at higher elevations (Kessler, 2001; Krömer et al., 2005; Linares-Palomino et al., 2009; Linares-Palomino and Kessler, 2009).

To understand the genesis of these patterns—and, more generally, the history of adaptive radiation and geographic diversification in bromeliads—we need a well-resolved, strongly supported phylogeny for this remarkable family. Progress toward this goal initially was slow, partly because bromeliads are taxonomically isolated, with no clear outgroup with which to polarize character-states (Gilmartin and Brown, 1987; Terry et al., 1997; Givnish et al., 2000; Pires and Sytsma, 2002); partly because bromeliad plastid DNA evolves at an unusually slow rate (Gaut et al., 1992, 1997; Givnish et al., 2004, 2005); and partly because previous studies had limited taxon sampling.

Over the last dozen years, however, these roadblocks have been mostly overcome, through a greater understanding of relationships among monocot families overall (Givnish et al., 2005; Chase et al., 2006; Graham et al., 2006) and, within Bromeliaceae, through the sequencing and analysis of one or a few rapidly evolving genes and gene spacers in the plastid genome by individual laboratories (e.g., Terry et al., 1997; Horres et al., 2000; Crayn et al., 2004; Givnish et al., 2004, 2007; Sass and Specht, 2010). Based on a thorough sampling of taxa in all three traditional subfamilies—especially the critical Pitcairnioideae (characterized by winged or unappendaged seeds)—Givnish et al. (2007) presented the most comprehensive view of bromeliad phylogeny and evolution to date, based on cladistic analyses of sequences of the plastid gene *ndhF* and calibration of the resulting molecular tree against the known ages of several monocot fossils. Their findings placed *Brocchinia*, then *Lindmania* at the base of the bromeliad family tree, sister to all other taxa. The upper branches of that tree consisted of a trichotomy including *Hechtia*, the subfamily Tillandsioideae (characterized by plumose seeds), and a "ladder" consisting of four clades embracing all other bromeliads, including *Puya* (part of the traditional Pitcairnioideae) as sister to Bromelioideae (characterized by fleshy fruits) (Fig. 2). Using this phylogeny, Givnish et al. (2007) erected a new, eight-subfamily classification for bromeliads, splitting the traditional but highly paraphyletic Pitcairnioideae into Brocchinioideae, Lindmanioideae, Hechtioideae, Navioideae, Pitcairnioideae s.s., and Puyoideae (Fig. 2). The *ndhF* phylogeny resolved more of the higher-level relationships in Bromeliaceae than studies including fewer genera based on *ndhF* (Terry et al., 1997), the *trnL* intron (Horres et al., 2000), or *matK* and *rps16* (Crayn et al., 2004), but was otherwise consistent with the results of those investigations. It also provided several new insights into the historical biogeography and adaptive radiation of bromeliads. However, the *ndhF* phylogeny provided only weak support for several nodes, failed to resolve the branching sequence of Tillandsioideae and Hechtioideae, and had a limited density of taxon sampling, including only 26 of 58 currently recognized genera, and none of the critical Chilean species of *Puya* (Jabaily and Sytsma, 2010) or Bromelioideae (Schulte et al., 2009).

To overcome these weaknesses, provide the basis for a more rigorous analysis of bromeliad evolution, and tap the wealth of data already in hand for several plastid loci—including those used to construct emerging, multilocus phylogenies for Bromelioideae (Schulte et al., 2005, 2009; Horres et al., 2007; Schulte and Zizka, 2008; Sass and Specht, 2010) and Tillandsioideae (Barfuss et al., 2005)—we formed an international consortium to produce a well-resolved, strongly supported phylogeny for Bromeliaceae based on multiple plastid loci and as comprehensive a sampling of bromeliad genera as could be managed.

Here we present the first results of that collaboration. To reconstruct relationships across Bromeliaceae, we completed the sequencing of eight rapidly evolving plastid regions for representatives of 46 of 58 bromeliad genera. We then used the resulting phylogeny to (1) analyze relationships within the family and test the new eight-subfamily classification, (2) infer the timing of divergence of various clades and relate these dates to events in Earth history, and (3) determine the geographical origins of the family and patterns of subsequent spread outside this region by members of each subfamily. A companion paper will calculate the rate of net species diversification for each major bromeliad clade and relate the observed differences in diversification rate to differences among clades in morphology, ecology, geographic disribution, mode of seed dispersal, and time of adaptive radiation.

MATERIALS AND METHODS

DNA extraction, taxon sampling, and selection of molecular markers—Total genomic DNAs were extracted using the protocols of Crayn et al. (2004), Barfuss et al. (2005), Schulte et al. (2005), and Givnish et al. (2007). We sequenced eight rapidly evolving plastid regions (*atpB-rbcL, matK, ndhF, psbA-trnH, rpl32-trnL, rps16, trnL* intron, *trnL-trnF*) for 90 bromeliad species representing 46 genera, and three outgroups from Rapateaceae and Typhaceae (Appendix 1). An 81-gene analysis of relationships among monocot families (Givnish et al., 2010) placed Bromeliaceae sister to all other families of the order Poales, with Typhaceae being sister to to all families of Poales except itself and Bromeliaceae, and Rapateaceae being sister to the remaining families of Poales. We used *Phoenix dactylifera* (Arecaceae) as the ultimate outgroup and downloaded sequences for all eight plastid regions for this species from the complete plastome sequence posted on GenBank.

Multiple species of *Aechmea*, *Mezobromelia*, *Navia*, *Ochagavia*, *Tillandsia*, and *Vriesea* were sampled due to concerns about the monophyly of those genera (Crayn et al., 2004; Barfuss et al., 2005; Schulte et al., 2005; Sass and Specht, 2010). Multiple species of *Brocchinia*, *Guzmania*, *Hechtia*, *Pitcairnia*, and *Puya* were included to help resolve the critical taxonomic positions of those genera. We included representatives of all genera of Brocchinioideae, Lindmanioideae, Tillandsioideae, Hechtioideae, Pitcairnioideae, and Puyoideae, all but one genus (*Steyerbromelia*) of Navioideae, and all but 11 of 34 genera of Bromelioideae (including 33 listed by Butcher 2008 and Luther 2008, and retaining *Pseudananas*). Of the 11 genera omitted, seven (*Androlepis*, *Fernseea*, *Hohenbergiopsis*, *Neoglaziovia*, *Orthophytum*, *Portea*, *Ursulaea*) were included in recent multilocus studies of relationships within Bromelioideae, and all were placed in that subfamily by plastid and nuclear data (Schulte and Zizka, 2008; Schulte et al., 2009; Sass and Specht, 2010). Genera not represented in this study include less than 2.5% of all described bromeliad species (see Luther, 2008). Subfamilial nomenclature follows Givnish et al. (2007).

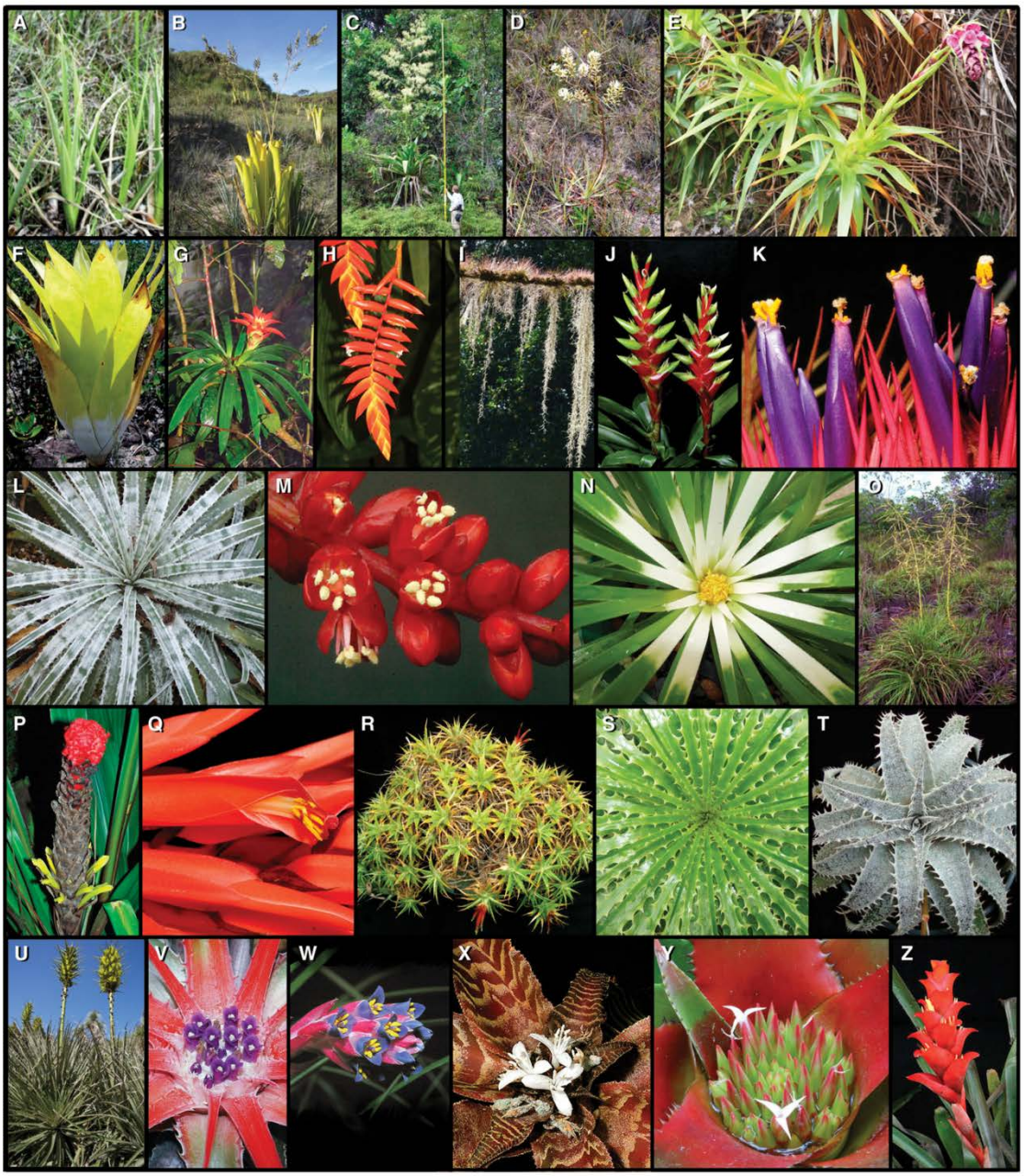

Fig. 1. Representative species of bromeliad subfamilies; images are at different scales. BROCCHINIOIDEAE: (A) *Brocchinia prismatica,* nonimpounding species sister to all *Brocchinia*, found in wet, sandy savannas in SW Venezuela; (B) *B. reducta*, terrestrial carnivore of damp, sandy savannas in SE Venezuela and SW Guyana; (C) tree-like *B. micrantha*, SE Venezuela and SW Guyana. LINDMANIOIDEAE: (D) *Lindmania guianensis*, SE Venezuela and SW Guyana; (E) *Connellia augustae,* sandstone outcrops, Venezuela and Guyana. TILLANDSIOIDEAE: (F) *Catopsis berteroniana*, carnivorous epiphyte, Florida to Brazil; (G) *Guzmania lingulata*, epiphyte, Central and N South America; (H) *Tillandsia dyeriana*, epiphyte, Ecuador; (I) *Tillandsia setacea* (above branch) and *T. usneoides* (Spanish moss, below branch), widespread atmospheric epiphytes; (J) *Vriesea heliconioides*, epiphyte, Mexico to

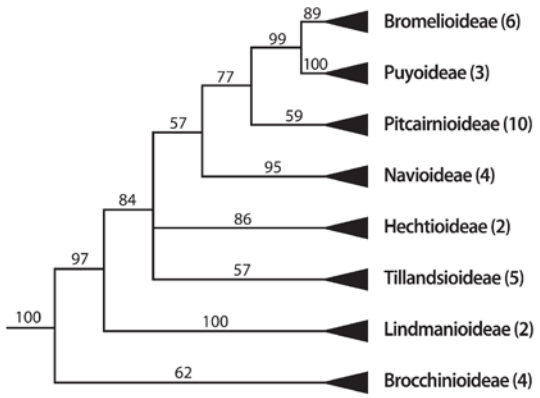

Fig. 2. Maximum-parsimony strict consensus tree from Givnish et al. (2007) based on variation in plastid *ndhF* sequences, with proposed relationships among bromeliad subfamilies. Outgroups from seven families of order Poales not shown. Numbers above branches are bootstrap support values; numbers in parentheses after subfamilial names indicate the number of taxa included in the earlier analysis.

We believe that our approach to higher-level bromeliad phylogenetics, based solely on sequences from the plastid genome, is justified because very few natural cases of hybridization among bromeliads are known, based on morphology or on more decisive comparisons of organellar vs. nuclear DNA markers (Wendt et al., 2008; Gonçalves and de Azevêdo-Gonçalves, 2009). Partly this may be because nuclear ribosomal ITS—the nuclear locus used to screen for hybridization and/or introgression in many angiosperm lineages—has only rarely been amplified and sequenced in bromeliads, given its strong hairpin geometry in this group (T. M. Evans, personal communication). However, Schulte et al. (2009), Gonsiska (2010), Jabaily and Sytsma (2010), and Sass and Specht (2010), employing other nuclear markers (*PhyC, PRK*, and nrDNA ETS) with plastid sequences to evaluate relationships among hundreds of species, have identified only a very small number of putative hybrids, most notably the ancestor of the Chilean clade of *Puya* and one species of *Catopsis.* Thus, here we rely on multiple loci from the plastome genome to reconstruct evolutionary relationships, recognizing that the validity of our plastid phylogeny should be tested when it becomes possible to sequence and align low-copy nuclear genes across all subfamilies.

DNA amplification, sequencing, and alignment—Methods for amplifying and cycle-sequencing different plastid regions from total DNA extracts followed Barfuss et al. (2005) for *atpB-rbcL* and *rps16;* Crayn et al. (2004) for *matK*; Givnish et al. (2007) for *ndhF*; Horres et al. (2000, 2007) for the *trnL* intron and *trnL-trnF*; and Shaw et al. (2007) for *psbA-trnH* and *rpl*32*-trnL.* Sequences were visually aligned following Baum et al. (1994). Stretches of DNA that were difficult to align (i.e., there were multiple conflicting alignments possible under the assumptions of Baum et al.) or had missing data for a substantial number of taxa were excluded from analysis. We were unable to complete 60 (9.2%) of 651 sequences. GenBank accession numbers were acquired for all new sequences; previously obtained sequences were downloaded from GenBank (Appendix 1). An aligned data set has been deposited in TreeBase (http://www.treebase.org/treebase-web/home.html; accessed 04-07-11), together with the maximum likelihood and Bayesian trees as case S11152.

Phylogenetic analyses—We inferred relationships from the nucleotide data using maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference (BI). MP analyses were conducted using the program PAUPRat (Sikes and Lewis, 2001), based on Parsimony Ratchet (Nixon, 1999) and implemented in the Cyberinfrastructure for Phylogenetic Research (CIPRES) portal 2 teragrid (http://www.phylo.org) (Miller et al., 2010). Individual bases were considered multistate, unordered characters of equal weight; unknown nucleotides were treated as uncertainties. Following Nixon (1999) and Goloboff (1999), we performed multiple (50) independent searches in PAUPRat to cover tree space adequately. Each search involved 500 iterations, with the shortest trees from each search used to form a strict consensus tree and a majority-rule tree. Shortest trees from each successive search were combined with previous search trees to evaluate whether the combined search consensus tree had stabilized. Stabilization of a consensus tree based on multiple, independent searches in PAUPRat supports the accuracy of the topology obtained (Goloboff, 1999). We used bootstrap analysis (Felsenstein, 1985) in the program PAUP* 4.0b10 (Swofford, 2002) to assess the relative support for each node in the strict consensus, using 1000 random resamplings of the data and retaining 200 trees per iteration. To determine the extent to which the lower support for the monophyly of Puyoideae and Bromelioideae in this study vs. Givnish et al. (2007) was due to our inclusion here of a number of Chilean *Puya* and Chilean bromelioids and *Deinacanthon* of the nearby Gran Chaco, respectively, we removed the latter from the analysis and recalculated support values for Puyoideae and Bromelioideae. Consistency indices, including autapomorphies (CI) and excluding them (CI′), were calculated to evaluate the extent of homoplasy in the data (Givnish and Sytsma, 1997). Maximum-parsimony phylogenies were also formed for each plastid region, and incongruence length difference (ILD) tests (Farris et al., 1994) were conducted for each pair of regions (*ndhF, matK, trnL-trnF, atpB-rbcL, psbA-trnH, rpl16, rpl32-trnL*) in PAUP* after removing taxa not sequenced for either region, to assess potential conflicts between regions in phylogenetic structure.

Maximum-likelihood analyses used the program jModelTest 0.1.1 (Posada, 2008) based on the program Phyml (Guindon and Gascuel, 2003) to select the appropriate model of nucleotide evolution using the Akaike information criterion (AIC) (Posada and Buckley, 2004). We evaluated models for each of the plastid regions separately and the entire concatenated sequence. The most likely tree was produced using the program GARLI (Genetic Algorithm for Rapid Likelihood Inference; Zwickl, 2006) in CIPRES. Multiple models for each gene partition are not allowed in GARLI, so the more complex model for a given set of genes was chosen. Maximum-likelihood bootstrapping (MLB) was completed using the program RAxML 7.0.4 (Stamatakis et al., 2005, 2008).

Bayesian inference was performed in the program MrBayes 3.1 (Ronquist and Huelsenbeck, 2003) allowing different models for each region. Four independent runs of 5 000 000 generations each were completed with a chain temp of 0.2. Trees were sampled every 1000 generations. The first 25% of runs were discarded as burn-in. A majority rule consensus of the remaining trees from the four runs was produced in PAUP* 4.0 and used as the Bayesian inference tree with posterior probabilities (PP). We also explored the mixture model of Pagel and Meade (2008) as implemented in the program BayesPhylogenies (Pagel and Meade, 2004). This model allows the fit of more than one model of evolution to each site in the alignment. We used the recommended GTR + Γ model with "patterns=2, pi=true", allowing two rate matrices to be formed and allowing both rate parameters and base frequencies to vary.

Dating radiations—An indirect approach to calibrating the bromeliad phylogeny is required because almost all bromeliads occur in habitats that are poor

←
Bolivia; (K) *Tillandsia ionantha*, flowers of tiny atmospheric epiphyte, Central America. HECHTIODEAE: (L) *Hechtia mooreana*, CAM terrestrial, Mexico; (M) partial inflorescence, *H. rosea*, CAM terrestrial, Mexico. NAVIOIDEAE: (N) *Navia* aff. *lactea*, saxicole, S Venezuela; (O) *Sequencia serrata*, E Colombia. PITCAIRNIOIDEAE: (P) *Pitcairnia holstii,* low-elevation terrestrial, Venezuela; (Q) bird-pollinated flowers, *P. undulata*, Mexico; (R) *Deuterocohnia lotteae*, high-elevation Andean cushion plant, S Bolivia; (S) *Encholirium spectabile*, CAM terrestrial, NE Brazil; (T) *Dyckia lindevaldae,* CAM terrestrial, Brazil. PUYOIDEAE: (U) *Puya chilensis*, tall terrestrial, Chile, cultivated at the Huntingdon Botanical Garden. BROMELIOIDEAE: (V) *Bromelia macedoi*, CAM terrestrial, Brazil; (W) *Fernseea bocainensis*, SE Brazil; (X) *Cryptanthus fosterianus*, nonimpounding CAM terrestrial, SE Brazil; (Y) *Neoregelia eleutheropetala* var. *bicolor*, CAM epiphyte with flowers emerging from tank, S tropical America; and (Z) *Canistrum alagoanum,* CAM epiphyte with flowers emerging from tank, SE Brazil. *Photo credits:* A, Thomas Givnish; B, Peggy Faucher; O, Julio Betancour; T, Reginaldo Baião; all others, Bruce Holst.

for fossil preservation. There is only one macrofossil clearly assignable to Bromeliaceae, from Costa Rica 36 million years ago (Ma) (Smith and Till, 1998), long after both existing estimates of the age of origin of Bromeliaceae based on molecular data (Givnish et al., 2004, 2007). Lemé et al. (2005) recently erected a new family for a bromeliad-like fossil (*Protananas lucenae*) from northeastern Brazil in limestone 100–110 Myr old. The authors report, however, that this taxon appears to be a nonbromeliad close to the base of order Poales.

We conducted two analyses to assess the timing of the rise of the bromeliad stem lineage within Poales and of the crown radiation of the family. First, building on previous monocot-wide analyses of relationships and fossil dating (Bremer, 2000; Givnish et al., 2000, 2005; Janssen and Bremer, 2004), we used *ndhF* sequences of 333 taxa of monocots (including 71 from Bromeliaceae) and the outgroup *Ceratophyllum* to build a monocot-wide phylogeny. The ML tree derived in GARLI using a model from jModelTest was used for subsequent fossil calibration. As *ndhF* alone does not have the power to resolve several key nodes, we constrained five areas of the monocot backbone based largely on the results of a recent monocot-wide study employing 81 plastid genes (Givnish et al., 2010). These constraints included (1) (Araceae, (Tofieldiaceae, all other Alismatales)); (2) (Liliales, (Asparagales + commelinids)); (3) (Dasypogonaceae, Arecaceae); (4) (Poales, (Commelinales, Zingiberales)); and (5) (Bromeliaceae, (Typhaceae, (Rapateaceae, all other Poales))). We used the Langley and Fitch (1974) method, as implemented in the program r8s (Sanderson 2004), to reconstruct divergence times on the ML tree with *Ceratophyllum* pruned off assuming a molecular clock and conduct a χ^2 test of rate constancy to test for significant deviation from clocklike evolution. Given the nonclocklike pattern of evolution observed, we converted the ML tree into ultrametric form using penalized likelihood (PL) in r8s (Sanderson, 2002, 2004), calibrated against monocot-wide fossils.

Six Cretaceous fossils were used to constrain the corresponding nodes as minimum ages (Janssen and Bremer, 2004; Givnish et al., 2005; Hesse and Zetter, 2007). The monocot root was fixed at 134 Ma (Bremer, 2000; Janssen and Bremer, 2004). Penalized likelihood smoothes local differences in the rate of DNA evolution on different branches, taking into account branch lengths and branching topology and assigning a penalty for rate changes among branches that are too rapid or frequent, based on a smoothness parameter. We used the cross-verification algorithm in r8s (Sanderson, 2004) to find the optimal value of the smoothness parameter, based initially on minimizing the sum of the squared deviations between the observed and expected branch lengths derived by jackknifing each branch (Sanderson, 2002). We varied the smoothness parameter from 10^{0} to 10^{3} in steps of 0.25 of the exponent. The optimal value of the smoothness parameter was validated using the check-gradient algorithm in r8s. We ran separate r8s analyses using a range of smoothness values near the optimum to examine the impact of different values on variation in the stem and crown age of Bromeliaceae and chose the final value of the smoothing parameter based on minimization of that variation within the window of values that yield similar, near-minimal sums of the squared deviations between observed and expected branch lengths (see above). To estimate uncertainties in node age due to uncertainties in the monocot-wide *ndhF* branching topology, we calculated the standard deviation of the estimated age for each node (including those within Bromeliaceae) by forming 100 bootstrap resamplings of the sequence data employing the program PHYLIP (Felsenstein, 1993) and then using these to calculate realized branch lengths of the original ML tree for each resampling. The optimal smoothness parameter obtained for the entire data set was used in calculations for each resampling.

Second, we conducted a detailed r8s analysis of the entire eight-locus Bromeliaceae data set (including *ndhF*) with *Rapatea* (Rapateaceae) and *Typha* and *Sparganium* (Typhaceae), as well as the ultimate outgroup *Phoenix* (Arecaceae). Although the monocot-wide *ndhF* phylogenetic and fossil-dating analyses included Bromeliaceae, the eight-locus data set is essential for obtaining a more finely resolved estimate of branching events and their timing within the family. The stem and crown dates of Bromeliaceae obtained from the fossil-calibrated *ndhF* monocot chronogram were used as fixed dates in r8s for the eight-locus ML tree after removing *Phoenix*. Due to the ambiguity of monophyly in *Puya* based on plastid data, but the compelling support for it from nuclear sequence data and morphology (Jabaily and Sytsma, 2010), we ran r8s analyses with *Puya* constrained to be monophyletic.

To estimate variation in node age due to uncertainties in the derived node dates of the eight-locus data set *and* in the *ndhF* stem and crown node dates, we performed three further analyses. First, we calculated the standard deviation of inferred age at each node via 100 bootstrap resamplings of the eight-locus data set. Second, we calculated the standard deviation of both the stem and crown nodal dates for Bromeliaceae based on 100 bootstrap resamplings of the monocot-wide *ndhF* data; this allowed us to generate of the mean ± SD of the inferred ages for both the stem and crown nodes based directly on fossil calibration. Given that variation in inferred node ages is a function of random variation in the ages of the set-dates independent of random variation in node ages due to uncertainty in the eight-locus phylogeny, an estimate of the total standard deviation of inferred age at the stem and crown nodes can be estimated as $SD_{total} = (SD^2_{set\text{-}dates} + SD^2_{phyl\ uncertainty})^{0.5}$ (see Givnish et al., 2009). Finally, to quantify any bias or degree of uncertainty resulting from using the stem and crown ages from the *ndhF* tree to calibrate the eight-locus tree, we regressed the stem and crown ages for several critical nodes (each subfamily; the core tillandsioids, *Navia/Brewcaria*, *Pitcairnia,* and the Brazilian Shield and epiphytic tank bromelioid clades [see Results]; Puyoideae + Bromelioideae; and Puyoideae + Bromelioideae + Pitcairnioideae) for the eight-locus tree on those for the *ndhF* tree, eliminating the stem age of Bromelioideae to avoid duplication.

We related the timing of inferred cladogenetic events to the times of uplift and dissection of the tepuis of the Guayana Shield, formation of the Amazon basin, uplift of the Andes and Brazil's Serra do Mar, and shifts in regional climate as estimated by a variety of geological, climatological, and biogeographic studies (e.g., Vasconcelos et al., 1992; Hoorn et al., 1995, 2010; van der Hammen, 1995; Amorim and Pires, 1996; Potter, 1997; Safford, 1999; Coltorti and Ollier, 2000; Gregory-Wodzicki, 2000; Auler and Smart, 2001; Behling and Negrelle, 2001; Wang et al., 2004; Grazziotin et al., 2006; Garzione et al., 2008; Antonelli et al., 2009; Ehlers and Poulsen, 2009; Figueiredo et al., 2009). Special attention was paid to the stem and crown ages of each subfamily, the core tillandsoids (sister to *Catopsis* and *Glomeropitcairnia*), and the clade of tank species sister to *Acanthostachys* (the core bromeliads; see Schulte et al., 2009).

Historical biogeography—To reconstruct spatial patterns of geographic diversification within Bromeliaceae, we employed three contrasting methods and accompanying assumptions implemented in the programs Statistical Dispersal–Vicariance Analysis (S-DIVA; Yu et al., 2010), BayesTraits (Pagel and Meade, 2007), and MacClade 4.08 (Maddison and Maddison, 2005). Given that the stem lineage of the family is already known to extend back to the Cretaceous but with a far more recent crown radiation (Givnish et al., 2004, 2007), and that bromeliads are clearly capable of long-distance dispersal—for example, from South America to the Galápagos (*Racinaea insularis*, Tillandsioideae), the Juan Fernandez Islands (*Greigia berteroi* and *Ochagavia elegans*, Bromelioideae), and tropical West Africa (*Pitcairnia feliciana*, Pitcairnioideae); see Smith and Downs (1974, 1977, 1979) and Givnish et al. (2007)—*any* assumption about the relative importance of vicariance vs. dispersal in Bromeliaceae would be difficult to justify. Programs to evaluate geographic diversification either favor vicariance (e.g., dispersal–vicariance analysis [DIVA, Ronquist, 1996, 1997; and S-DIVA]) or allow any amount of dispersal between areas (e.g., BayesTraits or MacClade using BI and MP criteria, respectively). Explicit, model-driven analyses of geographic diversification are possible (Ree et al., 2005; Ree and Smith, 2008), especially in the context of well-known geological events (e.g., continental vicariance as in Clayton et al., 2009), but remain premature for examining diversification within and among areas of geologically complex South America.

To minimize some of the shortcomings inherent in DIVA (Nylander et al., 2008; Harris and Xiang, 2009; Kodandaramaiah, 2010), we instead used S-DIVA (Yu et al., 2010). DIVA optimizes distributions for each node by allowing vicariance but minimizing assumptions of dispersal and extinction. S-DIVA extends DIVA by permitting assessment of phylogenetic uncertainty by examining multiple trees (in our case, a random subset of post burn-in Bayesian trees), each of which may contain polytomies.

Ranges of terminal taxa were atomized into recognized areas of endemism largely following Givnish et al. (2007) and (except for fusion of all Andean regions) Antonelli et al. (2009), including (1) Guayana Shield; (2) Brazilian Shield (including the Serra do Mar and Serra da Mantiqueira, as well as the adjacent Phanerozoic deposits of the Horn of Brazil and the Rio de la Plata basin); (3) Amazonia; (4) Caribbean (including the coast of northern South America and the southeastern United States); (5) Central America (including semiarid southern Texas); and (6) tropical West Africa. Distributional data were drawn from Smith and Downs (1974, 1977, 1979). Following the recommendation of Ronquist (1996), terminal species representing higher taxa (i.e., genera) were scored for ancestral area where possible (specifically, for *Catopsis* in Central America [Gonsiska, 2010]). When that approach was not justified or feasible, we scored single placeholders for all portions of the generic range (e.g., *Bromelia*) despite the known sacrifice in geographical resolution at deeper nodes in S-DIVA reconstructions (Ronquist, 1996). Multiple species per genus were each scored based on their own distribution. Vicariance between the Guayana Shield and the Andes, Caribbean, and Central America were excluded, as was

vicariance between tropical West Africa and any other region, due to the lack of any geographic contact between these regions over the inferred age of the bromeliad stem group. Due to the ancient split of Bromeliaceae from all other Poales, we performed several iterations of S-DIVA with respect to different outgroups (i.e., Rapateaceae and Typhaceae). Rapateaceae (and other lineages among the early splits in Poales) are Guayanan, whereas Typhaceae are cosmopolitan. We thus ran S-DIVA with the two outgroup families scored as Guayana Shield and polymorphic, respectively. We also ran analyses after scored both outgroups as Guayana Shield, due to the strong signal of Guayana Shield as basal in more Poales-wide biogeographic analyses (Givnish et al., 2000, 2004, 2007). Last, we removed Typhaceae entirely as an outgroup, as advocated by Bremer (2002), who removed this aquatic, easily dispersed group in DIVA analysis because it would be dangerous to base any conclusions regarding ancestral distributions on their present distributions. A random subset of 1000 Bayesian posterior probability trees from the phylogenetic analysis of the eight-locus data set were input into S-DIVA to estimate probabilities of ancestral areas at each node. We explored the impact of restricting the number of unit areas allowed in ancestral distributions by using the maxareas option (all possible areas, 4, and 2). The ancestral areas for all nodes were visualized on the ML tree with *Puya* constrained to be monophyletic.

We also analyzed the biogeographical data using ML and MP reconstructions that relax emphasis on vicariance by permitting dispersal between any pair of biogeographic areas. We implemented BI optimization of ancestral areas (Pagel, 1999) with the Markov chain Monte Carlo (MCMC)-based BayesMultiState option in the program BayesTraits v.1.0 (Pagel and Meade, 2007) using the ML tree with *Puya* constrained to be monophyletic to portray ancestral area reconstructions. To reduce some of the uncertainty and arbitrariness of choosing priors under MCMC, we used the hyperprior approach (the rjhp command) as recommended (Pagel et al., 2004; Pagel and Meade 2007). Combinations of hyperprior values (exponential or gamma, mean and variance) and rate parameter values were explored to find acceptance rates when running the Markov chains of between 20 and 40% (as recommended by Pagel and Meade, 2007). All subsequent analyses used the reversible-jump hyperprior command (rjhp gamma 0 30 0 10) that seeded the mean and variance of the gamma prior from uniform hyperpriors on the interval 0 to 30 and 0 to 10, respectively, and a rate parameter of 150 (ratedev 150). We reconstructed ancestral areas using MP by overlaying the ranges of individual species (or inferred ancestral area for *Catopsis*) using MacClade 4.08 (Maddison and Maddison, 2005), resolving all of the most parsimonious states at each node of the ML tree.

RESULTS

Phylogeny—We obtained an aligned data matrix of 94 taxa × 9341 characters; of the latter, 1210 were parsimony-informative and 1429 were variable but parsimony-uninformative (Table 1). The number of informative characters varied nearly 6-fold among loci, from 61 for *psbA-trnH* to 357 for *ndhF*. The fraction of informative sites varied from 8.8% (*psbA-trnH*) to 16.2% (*rpl32-trnL*). The numbers of informative vs. variable but uninformative characters were strongly correlated with each other across loci ($r = 0.97$, $P < 0.0001$ for two-tailed t test with 6 df), and the ratio of informative to variable but uninformative characters averaged 0.85 ± 0.074 (mean ± SD). Within Bromeliaceae, 1663 characters were variable, of which 766 were informative.

Maximum parsimony resulted in a single island of 1 317 600 trees of length 4546 steps, and a strict consensus tree that was well resolved outside subfamily Bromelioideae (Fig. 3). The consistency index CI for these trees was 0.70; CI′ (excluding autapomorphies) was 0.54. Branches that were unusually short (see below) were usually lost in the strict consensus tree relative to the majority-rule tree (Fig. 3).

The MP strict consensus tree supported the monophyly of all eight proposed subfamilies; each had 99–100% bootstrap support except Puyoideae and Bromelioideae (Fig. 3). Chilean *Puya* formed a clade with 100% bootstrap support; non-Chilean *Puya* had 99% support. *Puya* as whole—while resolved as monophyletic—had less than 50% support (Fig. 3). Bromelioideae had 59% bootstrap support. *Bromelia*, *Fascicularia-Ochagavia*, *Deinacanthon*, and *Greigia* formed a weakly supported clade sister to all other bromelioids in the MP majority-rule tree and a basal polytomy in the strict consensus tree. *Pseudananas* is sister to the remaining bromelioids (61% bootstrap), then *Ananas*. A core group of bromelioids, sister to and including *Ananas,* had 88% bootstrap support, but seven of 24 relationships within this core group were unresolved in the strict consensus (Fig. 3). The clade consisting of Bromelioideae and Puyoideae had 100% bootstrap support.

Support levels for the monophyly of each of the eight subfamilies in the strict consensus tree were generally much higher than those in the original *ndhF* phylogeny (Figs. 2, 3), except for Puyoideae and Bromelioideae. Experimental removal of taxa show that these two subfamilies had lower support in the curent analysis due to our inclusion of Chilean *Puya*, Chilean bromelioids, and *Deinacanthon* from the nearby Gran Chaco. Relationships among the eight subfamilies agreed with those in the original *ndhF* phylogeny (Fig. 2) but were better supported. In addition, the eight-locus data set resolved the subfamilial trichotomy present in the *ndhF* phylogeny, placing Hechtioideae sister to (Navioideae, (Pitcairnioideae, (Bromelioideae, Puyoideae))), and Tillandsioideae sister to all five subfamilies (Fig. 3).

In both the strict consensus and majority-rule trees, *Brocchinia*, *Guzmania*, *Hechtia*, *Deuterocohnia*, *Dyckia*, *Encholirium*, *Fosterella*, *Pitcairnia*, *Puya*, *Ananas*, and *Araeococcus* emerged as monophyletic. In contrast, *Lindmania*, *Tillandsia*, *Navia*, and *Ochogavia* were paraphyletic; *Mezobromelia*, *Vriesea*, and especially *Aechmea* (with at least six apparent "origins") were polyphyletic (Fig. 3). In the MP majority-rule tree, *Acanthostachys* was sister to taxa corresponding to the tank-bromelioid clade ("core bromelioids") of Schulte et al. (2009) and its sister *Cryptanthus*; *Acantostachys*, *Cryptanthus*, and the tank bromelioids formed an unresolved trichotomy in the strict consensus (Fig. 3).

MP trees based on individual plastid regions were less resolved and less well supported than the strict consensus phylogeny

TABLE 1. Numbers of parsimony-informative, variable but parsimony-uniformative, and invariant sites for each of the plastid regions sequenced, as well as the consistency indices (with and without autapomorphies) and proportion of informative sites for those regions.

Region:	*matK*	*ndhF*	*rps16*	*atpB-rbcL*	*psbA trnH*	*rpl32- trnL*	*trnL-trnF, trnL* intron	Total
No. informative sites	213	247	132	123	70	195	169	1149
No. variable but uninformative sites	200	310	151	145	71	251	170	1298
No. invariant sites	1218	1541	862	1109	759	937	808	7234
Total aligned bp	1631	2098	1145	1377	900	1383	1147	9681
Consistency index (CI)	0.70	0.71	0.72	0.66	0.69	0.72	0.73	0.71
C'	0.56	0.54	0.57	0.49	0.55	0.56	0.59	0.55
Informative sites/base	0.131	0.118	0.115	0.089	0.078	0.140	0.141	0.119

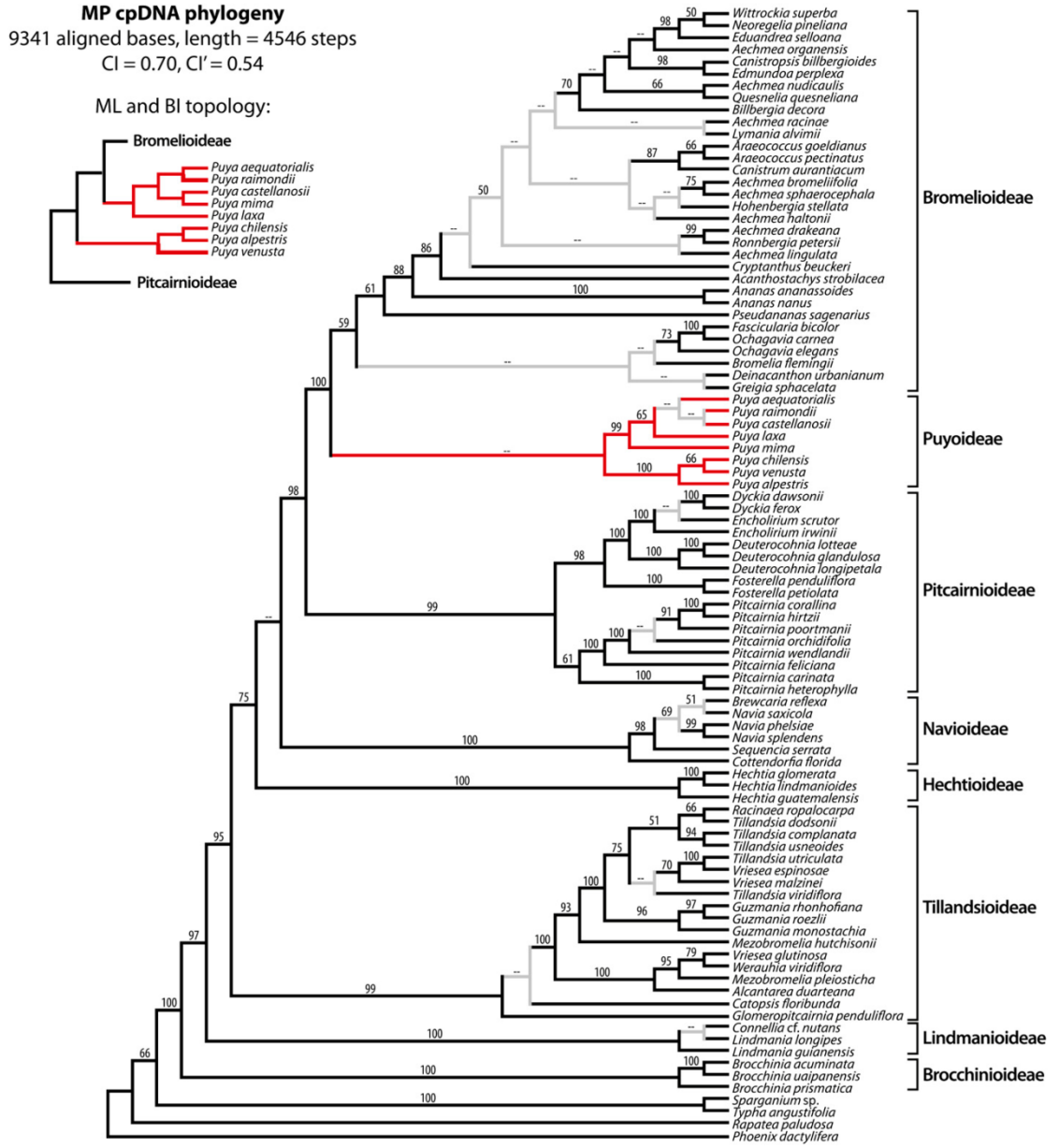

Fig. 3. Maximum-parsimony (MP) majority-rule phylogeny based on eight plastid loci; figure also shows the MP strict consensus tree, in which the light gray branches collapse. Numbers above branches are bootstrap support values; missing values indicate support less than 50%. Tree length = 4546 steps; CI = 0.70 and CI′ = 0.54 excluding autapomorphies. *Puya* (red branches) is monophyletic in the MP tree, but paraphyletic in the maximum-likelihood (ML) and Bayesian inference (BI) trees (see inset).

based on the combined data set. Although ILD tests showed apparently significant differences in phylogenetic structure between some pairs of regions, such differences only occurred in comparisons when one or both regions with relatively small numbers of phylogenetically informative sites (Table 1). Furthermore, for each region, the MP strict-consensus tree did not

diverge from the combined-data phylogeny at nodes well supported (≥90% bootstrap support) in the former.

For maximum-likelihood analysis, the AIC identified the optimal models as TVM + Γ for *ndhF*; TVM + I + Γ for *matK*, *trnL* (plus intron), *atpB*, and *rps16*; and GTR + I + Γ for *psbA-trnL* and *rpl32*. The maximum-likelihood and Bayesian trees were nearly identifical to each other in topology and mostly congruent with the MP majority-rule tree, but placed Bromelioideae in a paraphyletic *Puya,* sister to the non-Chilean taxa (Figs. 3–5). Both ML and BI placed *Hechtia* sister to Navioideae-Pitcarnioideae-Puyoideae-Bromeliodeae, congruent with the MP tree. Both placed *Catopsis* sister to *Glomeropitcairnia* at the base of the tillandsioids (Figs. 4, 5). The four areas of greatest phylogenetic uncertainty within bromeliads—as judged by differences in topology among trees or the degree of resolution within each tree—correspond to the portions of those trees with exceedingly short branch lengths, including (1) early-divergent bromelioids, (2) late-divergent bromelioids, (3) relationships among Chilean and non-Chilean *Puya*, and (4) relationships among *Catopsis*, *Glomeropitcairnia*, and all other tillandsioids (Figs. 3, 5). Conflicts among the three phylogenies generally did not occur at nodes that are well supported by each individually.

Molecular clocks and dating—Cross-verification of a penalized-likelihood calibration of the *ndhF* ML tree across monocots showed that smoothing parameters between 50 and 100 yielded very similar, nearly minimal sums of the squared deviations between the observed and expected branch lengths derived by jackknifing each branch. Within that range, a smoothing parameter of 75 minimized the variance in the apparent ages of the crown and stem node of Bromeliaceae. We used this value to calibrate the across-monocot tree, producing estimates of the bromeliad stem age as 100.0 ± 5.2 million years ago (Ma) (and the corresponding crown age as 19.1 ± 3.4 Ma (Fig. 6). These dates were then employed to calibrate the eight-locus bromeliad tree; cross verification produced a smoothness parameter of 100. The resulting chronogram (Fig. 7) resolved cladogenetic events within Bromeliaceae from 19.1 to 0.64 Ma. The standard deviation of estimated ages for individual nodes generally varied from 0.5 to 2 Myr, with smaller estimated amounts of variation due to phylogenetic uncertainty in nodes closer to the present (Fig. 7). Regression of estimated ages for several representative nodes in Bromeliaceae from the eight-locus tree on those from the across-monocots phylogeny (Table 2) yielded excellent agreement between the two sets of estimates ($y = 1.060x - 0.032$, $r^2 = 0.80$, $P < 0.0001$ for 25 df).

Historical biogeography—Reconstruction of ancestral areas using MP, BI, and S-DIVA generally agreed with each other, with the exception of a few nodes detailed below (Fig. 8). Based on our eight-locus chronogram and biogeographic reconstruction using MP, we infer that bromeliads arose in the Guayana Shield ca. 100 Ma, based on the restriction to this ancient craton—and in most cases, to highly leached marine sandstones of the overlying Precambrian Roraima Formation—of Brocchinioideae and Lindmanioideae, nested sequentially at the base of the family. Brocchinioideae diverged from the ancestor of all other bromeliads ca. 19.1 Ma, and extant species of *Brocchinia* began to diverge from each other ca. 13.1 Ma (Fig. 8). All other extant bromeliad subfamilies began diverging from each other slightly before that, with the stem lindmanioids diverging from the ancestor of other bromeliads ca. 16.3 Ma. The stem tillandsioids arose shortly after that, ca. 15.4 Ma (Fig. 8). Based on MP, it is unclear whether tillandsioids arose on the northern littoral of South America, in the Andes, or in Central America (Fig. 8). *Catopsis,* sister to *Glomeropitcairnia* with it sister to the remaining tillandsioids, today grows in the Guayana Shield as well as the north coast of South America, the Caribbean, Central America, and southern Florida, but appears to have arisen in Central America (Fig. 8). *Glomeropitcairnia* is endemic to the Lesser Antilles, Trinidad, and Tobago, and the north coast of Venezuela, and appears to have diverged from *Catopsis* about 14.0 Ma. The ancestor of the remaining members of the subfamily—which we term the core tillandsioids—appears to have arisen in the Andes about 14.2 Ma, with the modern genera beginning to diverge from each other ca. 8.7 Ma, with evolution mainly in the Andes but with several subsequent invasions of Central America, the northern littoral of South America, and the Caribbean (Fig. 8).

Hechtia arose ca. 16.6 Ma and invaded Central America independently (Fig. 8). Extant species of *Hechtia* began differentiating from each other ca. 10.3 Ma. About 15.0 Ma, Navioideae arose in the Guayana and/or Brazilian Shields, with restriction to the Guayana Shield after 10.4 Ma, corresponding to the endemism there of *Brewcaria*, *Navia*, and *Sequencia* and of *Cottendorfia* to the Brazilian Shield.

The common ancestor of the three remaining subfamilies evolved about 15.0 Ma in the Andes (Fig. 8), where *Pitcairnia* grows from near sea level to above treeline (with scattered occurrences elsewhere in the Guayana Shield and southeastern Brazil), *Fosterella* grows mostly at midelevations in mesic sites (with disjunct occurrences in Central America), *Dyckia* grows in drier sites from mid to high elevations and extends into the Brazilian Shield and the Rio de la Plata basin (including the Gran Chaco within the latter), and *Deuterocohnia* occurs as cushion plants in arid, high-elevation sites just south of the "knee" of the Andes, in southern Bolivia and northern Argentina (Fig. 9). Pitcairnioideae arose ca. 13.4 Ma; *Pitcairnia*, ca. 12.0 Ma; *Fosterella*, ca. 11.3 Ma; and *Deuterocohnia,* ca. 8.5 Ma. Based on the taxa included in this study, the lineage leading to *Pitcairnia feliciana* dispersed to Guinea in west Africa from the Andes sometime in the last 9.3 Myr. *Dyckia* and *Encholirium* (the latter restricted to northeastern Brazil) form a clade sister to *Deuterocohnia* and apparently invaded the Brazilian Shield from the Andes, beginning 8.5 Ma (Figs. 8, 9). Given the geographic overlap of *Deuterocohnia*, *Dyckia*, and *Fosterella* in south-central Bolivia (Fig. 9), it is likely that key cladogenetic events in Pitcairnioideae occurred there.

The common ancestor of *Puya* and the bromelioids arose about 13.4 Ma in the Andes (Fig. 8). Ancestral *Puya* diverged from the ancestral bromelioids ca. 10.1 Ma, with *Puya* splitting almost immediately (10.0 Ma) into two clades distributed in the Andes in low-elevation Chile vs. the rest of the cordillera at mid to high elevations. Present-day species of *Puya* began to diverge from each other during the last 3.5 Myr in the Andes, and during the last 2.5 Myr in Chile (Fig. 8). In the ML, BI, and MP majority-rule trees, a clade of five small bromelioid genera—mostly from Chile and the southern Andes—are sister to the remaining members of Bromelioideae (Fig. 8). Three of these genera (*Fascicularia-Ochagavia* and *Greigia*) are partly or wholly restricted to temperate regions at low elevations in the southern Andes, including low-elevation habitats just above high tide in *Fascicularia bicolor* and *Ochagavia litoralis* in continental Chile, and *O. elegans* in the Juan Fernandez Islands. *Greigia* grows in montane habitats from Central America to the Andes, and in the

Fig. 4. Maximum-likelihood (ML) phylogram for Bromeliaceae based on concatenated sequenced data. Branch lengths are proportional to the inferred number of nucleotide changes down each branch. *Puya* (red branches) in paraphyletic in the ML tree, but monophyletic in the MP tree.

understory of humid deciduous and evergreen forests in southern Chile and the offshore Juan Fernandez Islands. Two other genera—monotypic *Deinocanthon* and species-rich *Bromelia*—grow in the Gran Chaco (the southwestern portion of the Rio de la Plata basin, adjacent to the Andes) and throughout the Neotropics at low elevations, respectively (Fig. 8).

The remaining bromelioids form the "Brazilian Shield clade", which arose in the Brazilian Shield ca. 10.1 Ma via dispersal from the Andes (Fig. 8). Members of this clade subsequently dispersed repeatedly outside this region, notably in *Ananas*, *Aechmea*, *Araeococcus*, *Billbergia*, *Neoregelia*, and *Ronnbergia*, but most taxa are restricted to a narrow portion of the Brazilian Shield near the southeastern coast of Brazil, running ca. 1500 km from Minas Gerais to Rio Grande do Sol. This area includes the Brazilian Highlands (Serra do Mar and the more inland Serra da Mantiqueira) and adjacent coastal plain, with their extremely humid, highly diverse Atlantic rain forests and cloud forests, restingas on sandy soils, mangroves, campos de altitude, and drier vegetation inland (e.g., campos rupestres on rocky outcrops). The bromelioid tank-epiphyte clade—sister to

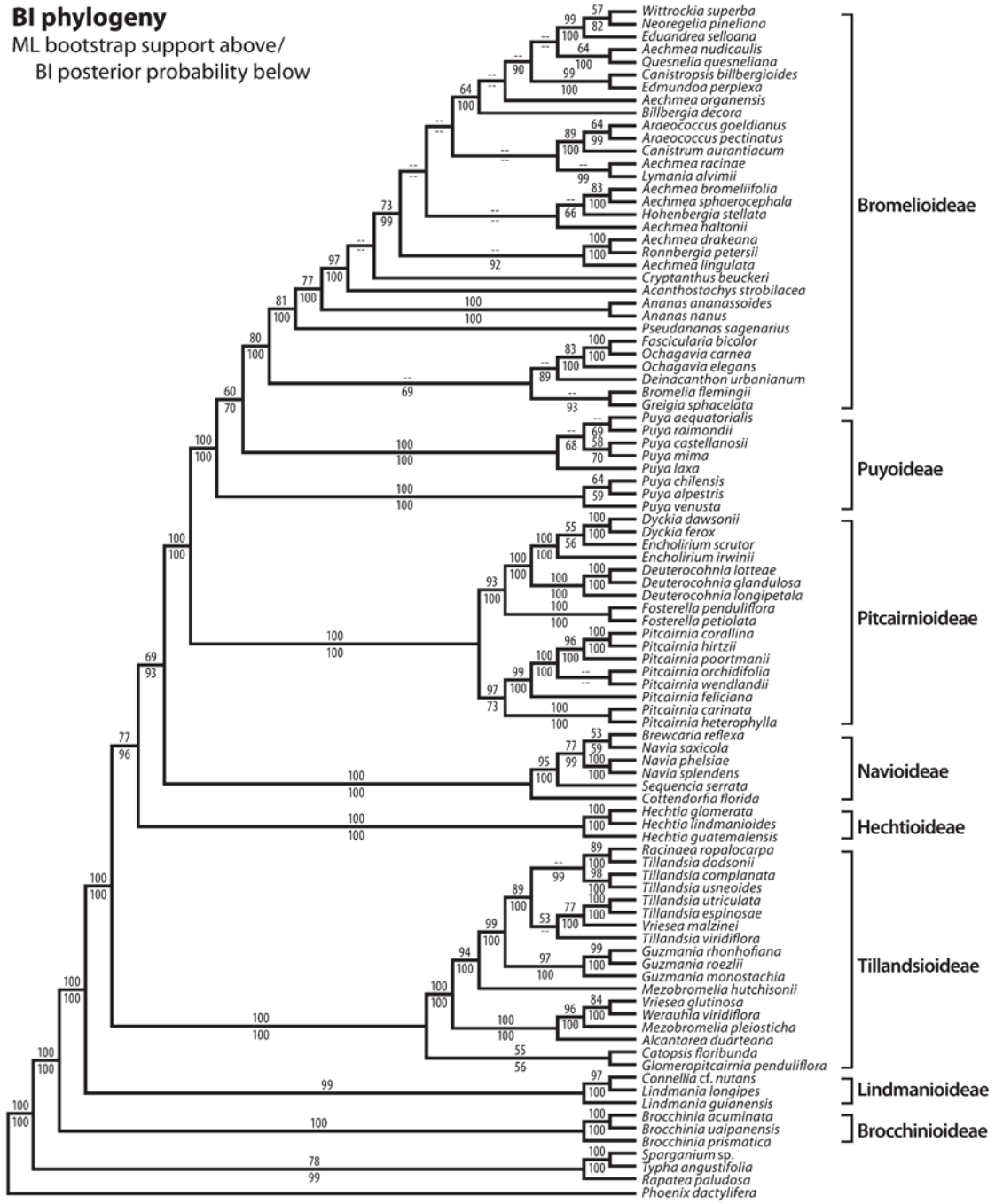

Fig. 5. Bootstrap support values (above each branch) and posterior probabilities (below each branch) for the maximum-likelihood/Bayesian inference tree.

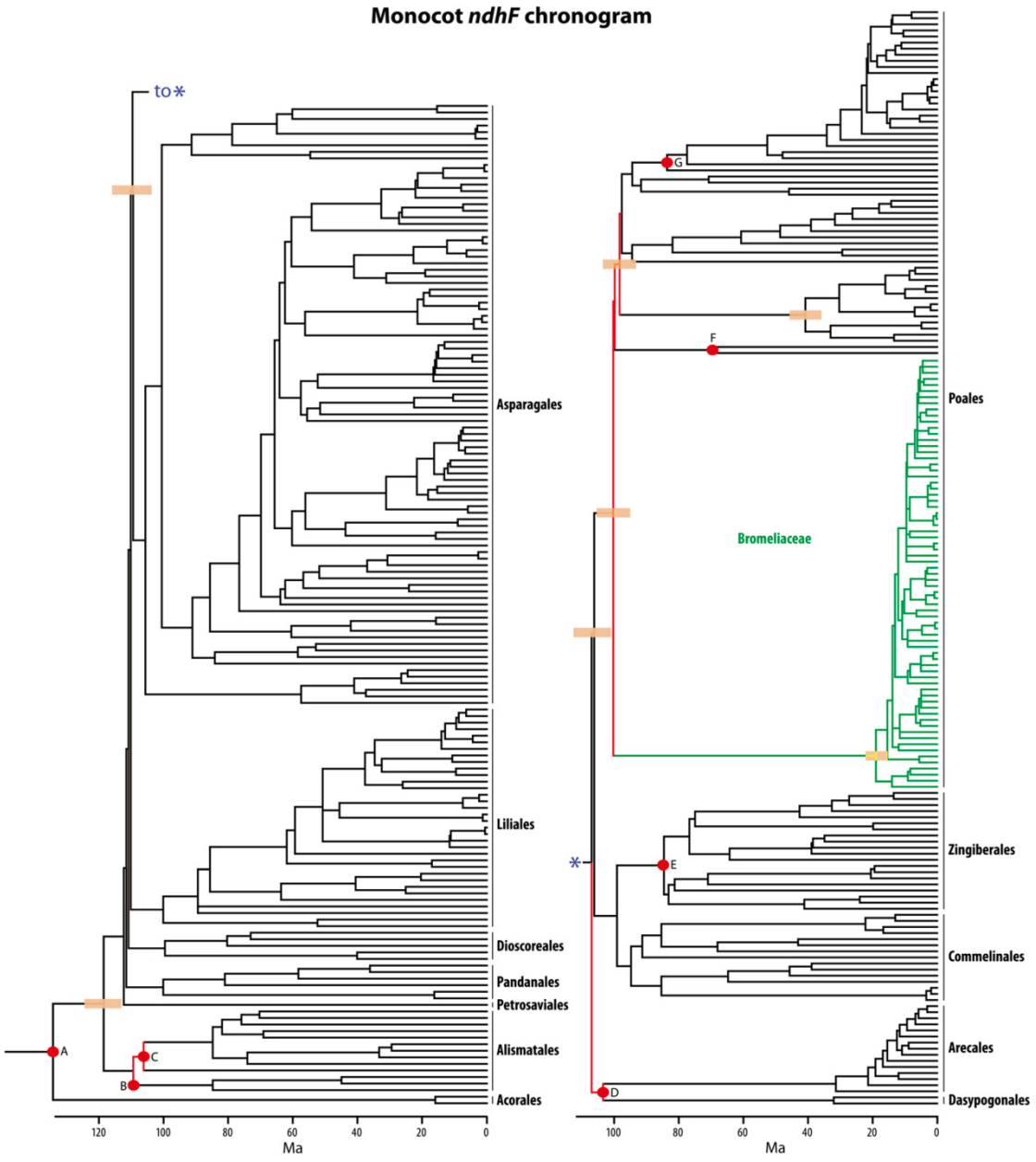

Fig. 6. Cross-verified penalized-likelihood chronogram across monocots based on the maximum-likelihood analysis of *ndhF* sequence variation. A = age of monocot root = 134 Ma (Janssen and Bremer, 2004): B–G = ages of the six Cretaceous fossils (Givnish et al., 2004; Janssen and Bremer, 2004) used to calibrate the monocot phylogeny against time. Bromeliaceae are highlighted in green. Tan boxes indicate ±1 SD, based on bootstrap resamplings, around the estimated ages of several key nodes (red dots), including the core monocots (excluding Acorales and Alismatales), commelinid monocots, order Poales, families Bromeliaceae and Rapateaceae, and remaining Poales sister to Rapateaceae. Red branches indicate those whose topology was constrained based on the plastome tree of Givnish et al. (2010).

Cryptanthus-Acanthostachys—is nearly restricted to this region and arose 9.1 Ma, with present-day taxa diverging from each other ca. 5.5 Ma (Fig. 8).

Reconstruction of the geographic spread of bromeliads under Bayesian inference tells largely the same story. Bayesian inference is, however, somewhat more specific than maximum parsimony about the likely origins of the tillandsioids and navioids. This portion of the tree is the largest that is not fully resolved biogeographically under MP, involving the rapid-fire divergence of four major lineages between 15.4 and 15.0 Ma, and accounting today for all but 2% of all bromeliad species. Bayesian inference reconstructed this portion of the bromeliad spine as being most probably Andean in origin (Fig. 8). Together with the BI reconstruction of the distribution of the stem tillandsioids and navioids, this suggests that tillandsioids arose in the Andes with many subsequent dispersals to other regions, especially Central America, the northern littoral of South America, and the Caribbean. It also suggests that ancestral navioids were, at some point, restricted to the Guayana Shield, with later dispersal or vicariance leading to occupancy of the Brazilian Shield by *Cottendorfia* (Fig. 8). BI suggests that the Guayana Shield or the Andes characterized the stem group for all bromeliads except Brocchinioideae and Lindmanioideae. Maximum parsimony instead points to this group's origin—as well as that of the common ancestor of Hechtioideae and its sister group—being in the Guayana Shield, Andes, or Central America. Maximum parsimony identifies these three areas, as well as the northern littoral of South America and the Caribbean, as possible ancestral areas for Tillandsioideae and *Catopsis-Glomeropitcairnia* (Fig. 8). Maximum parsimony identifies the Guayana Shield, Brazilian Shield, Andes, and Central America as possible ancestral areas for Hechtioideae and the common ancestor of Hechtioideae and the subfamilies to which it is sister. Bayesian inference is less certain than MP in reconstructing the biogeographic origins of *Pitcairnia*, assigning it to one of five areas while MP assigns it to the Andes. Bayesian inference is also less certain than MP in reconstructing the ancestral area of *Bromelia* and *Greigia,* making it equally likely that their common ancestor arose in Central America, the northern littoral of South America and the Caribbean, or the Andes. Bayesian inference reconstructs the stem region of Bromelioideae as being nearly equally likely to be the Andes or Brazilian Shield, with the taxa in the clade sister to the Brazilian Shield clade all being native to the southern Andes/Chile and the Gran Chaco, in the extreme southwest of the Rio de la Plata basin.

Finally, when outgroups are excluded, S-DIVA implies that the Guayana Shield is the ancestral area for Bromeliaceae, Brocchinioideae, and Lindmanioideae (Fig. 8). S-DIVA estimates the chance that the ancestral area for Tillandsioideae is the northern littoral of South America or Caribbean as 29%; that area fused to the Andes, 31%; and that same area fused to Central America, 40%. The chance that the ancestor of Tillandsioideae and its sister groups arose in the Guayana Shield fused to the northern American littoral and Caribbean is 31%; in the Andes alone, 33%; and in Central America alone, 36%. *Catopsis-Glomeropitcairnia* originated in Central America fused to the northern littoral of South America and Caribbean (Fig. 8). S-DIVA identifies the Andes fused to Central America as the ancestral area for Hechtioideae and its sister clade and the ancestral area of Navioideae and its sister clade as the Andes fused to the Brazilian Shield. Under this approach, Navioideae arose in the Guayana Shield fused to the Brazilian Shield, while the extant bromelioids arose in the Andes fused to the Brazilian Shield (Fig. 8). At other nodes, S-DIVA without outgroups usually reconstructs the same ancestral areas as MP and BI, except for *Pitcairnia,* which it implies arose in the Andes. Including outgroups changed the S-DIVA reconstruction little except at the base of Bromeliaceae, where a greater range of possible source regions were identified.

DISCUSSION

Phylogenetic relationships—Our analysis—based on more sequence data per taxon and wider sampling of genera than any previous study—supports the eight-subfamily classification advanced by Givnish et al. (2007) based on *ndhF* sequences (Fig. 2), and further clarifies the relationships among those subfamilies (Figs. 3–5). In the MP strict consensus, six subfamilies received bootstrap support ≥96%. Bromelioideae had 55% bootstrap support; Puyoideae, <50%. Support for five subfamilies increased relative to the *ndhF* study, but that for Lindmanioideae, Puyoideae, and Bromelioideae decreased as a result of the greater breadth of taxonomic sampling, including *Connellia,* the three Chilean *Puya* species, and several Chilean bromelioids. When we excluded the latter from our analysis, bootstrap support for both Puyoideae and Bromelioideae jumped to 100%; when we excluded *Connellia*, support for Lindmanioideae also reached 100%.

The MP, ML, and BI trees all support a stepped phylogeny for the bromeliad subfamilies: (Brocchinioideae, (Lindmanioideae, (Tillandsioideae, (Hechtioideae, (Navioideae, (Pitcairnioideae, (Puyoideae, Bromelioideae))))))). In Givnish et al. (2007), *Hechtia* instead formed a hard trichotomy with Tillandsioideae and all subfamilies sister to and including Navioideae. Our results clarify the position of Hechtioideae and, thus, the relationships of all bromeliad subfamilies. Support for the position of Navioideae is less than 50% under maximum parsimony, compared with 69% under maximum likelihood and 93% under Bayesian inference (Fig. 5).

Our results concur with the general finding that tree resolution and support for most angiosperm clades increase in combined vs. separate plastid gene analyses (e.g., Soltis et al., 1998, 2000; Savolainen et al., 2000; Olmstead et al., 2000, 2001; Bremer et al., 2002; Chase et al., 2006; Graham et al., 2006). Furthermore, simulations show that phylogenetic resolution and support can also improve with more taxa sampled within a given clade (Hillis, 1996; Graybeal, 1998), particularly when taxa are added strategically to break up long branches (Hendy and Penny, 1989; Leebens-Mack et al., 2005). While a number of ILD tests suggest that some plastid regions sequenced in this study show conflict in phylogenetic structures, we believe that this conflict is illusory. First, the plastid genome is inherited as a unit, so individual plastid regions should not conflict in the phylogenetic history their sequences reflect (Doyle, 1992). Second, trees based on each individual region generally do not differ from the combined-data phylogenies at nodes resolved and well supported in the individual-region trees. However, it must be realized that the limited number of informative sites in several data partitions (Table 1) result in few resolved and well-supported nodes in many individual-region trees. For example, we found that sequences for *atpB-rbcL* resolve only 40% of the nodes within Bromeliaceae; of those, 63% have bootstrap support from 50 to 90%, and only 21% (8 nodes) have bootstrap values greater than 90%. The whole point of concatenating plastid data are that individual genes and spacers each contain

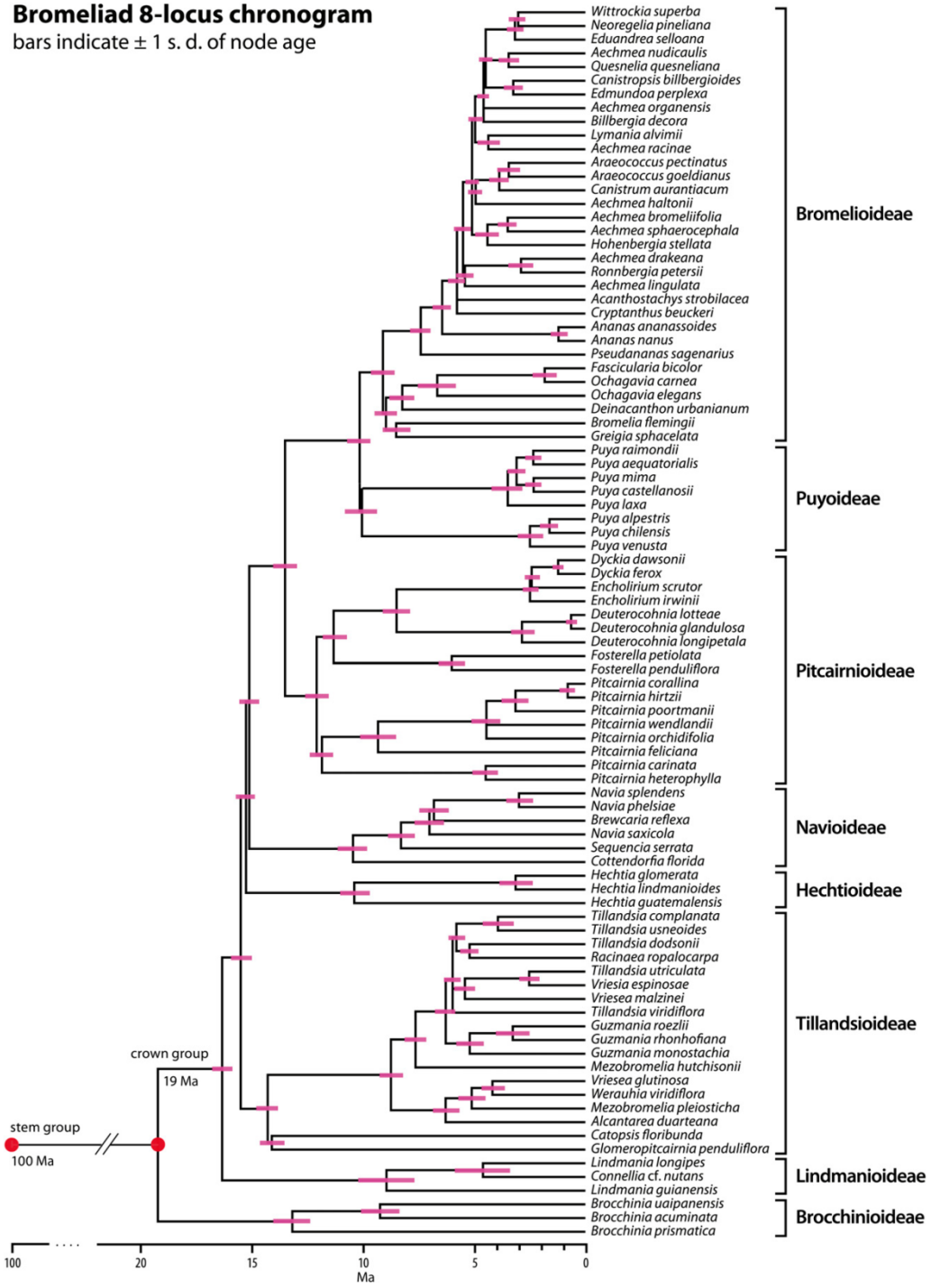
Bromeliad 8-locus chronogram
bars indicate ± 1 s. d. of node age
Wittrockia superba
Neoregelia pineliana
Eduandrea selloana
Aechmea nudicaulis
Quesnelia quesneliana
Canistropsis billbergioides
Edmundoa perplexa
Aechmea organensis
Billbergia decora
Lymania alvimii
Aechmea racinae
Araeococcus pectinatus
Araeococcus goeldianus
Canistrum aurantiacum
Aechmea haltonii
Aechmea bromeliifolia
Aechmea sphaerocephala
Hohenbergia stellata
Aechmea drakeana
Ronnbergia petersii
Aechmea lingulata
Acanthostachys strobilacea
Cryptanthus beuckeri
Ananas ananassoides
Ananas nanus
Pseudananas sagenarius
Fascicularia bicolor
Ochagavia carnea
Ochagavia elegans
Deinacanthon urbanianum
Bromelia flemingii
Greigia sphacelata
Puya raimondii
Puya aequatorialis
Puya mima
Puya castellanosii
Puya laxa
Puya alpestris
Puya chilensis
Puya venusta
Dyckia dawsonii
Dyckia ferox
Encholirium scrutor
Encholirium irwinii
Deuterocohnia lotteae
Deuterocohnia glandulosa
Deuterocohnia longipetala
Fosterella petiolata
Fosterella penduliflora
Pitcairnia corallina
Pitcairnia hirtzii
Pitcairnia poortmanii
Pitcairnia wendlandii
Pitcairnia orchidifolia
Pitcairnia feliciana
Pitcairnia carinata
Pitcairnia heterophylla
Navia splendens
Navia phelsiae
Brewcaria reflexa
Navia saxicola
Sequencia serrata
Cottendorfia florida
Hechtia glomerata
Hechtia lindmanioides
Hechtia guatemalensis
Tillandsia complanata
Tillandsia usneoides
Tillandsia dodsonii
Racinaea ropalocarpa
Tillandsia utriculata
Vriesia espinosae
Vriesea malzinei
Tillandsia viridiflora
Guzmania roezlii
Guzmania rhonhofiana
Guzmania monostachia
Mezobromelia hutchisonii
Vriesea glutinosa
Werauhia viridiflora
Mezobromelia pleiosticha
Alcantarea duarteana
Catopsis floribunda
Glomeropitcairnia penduliflora
Lindmania longipes
Connellia cf. nutans
Lindmania guianensis
Brocchinia uaipanensis
Brocchinia acuminata
Brocchinia prismatica
Bromelioideae
Puyoideae
Pitcairnioideae
Navioideae
Hechtioideae
Tillandsioideae
Lindmanioideae
Brocchinioideae
crown group
19 Ma
stem group
100 Ma
100
20
15
10
5
0
Ma

TABLE 2. Stem and crown ages of bromeliad subfamilies and subsets thereof, based on penalized-likelihood analyses of the across-monocots *ndhF* tree and the eight-locus plastid phylogeny.

	Stem age (Myr)		Crown age (Myr)	
Taxon	*ndhF*	8-locus	*ndhF*	8-locus
Brocchinioideae	19.1	19.1	14.2	13.1
Lindmanioideae	15.6		16.3	8.9
Tillandsioideae	14.0	15.4	11.8	14.2
Core tillandsioids	11.8	14.2	6.8	8.7
Hechtioideae	14.0	15.2	12.1	10.3
Navioideae	14.0	15.0	9.4	10.4
Navia-Brewcaria	9.3	8.3	8.6	7.0
Pitcairnioideae	13.3	13.4	9.4	11.8
Pitcairnia	13.2	12.0	9.4	11.8
Puyoideae	9.8	10.1	8.7	10.0
Bromelioideae	9.8	10.1	9.5	8.9
Brazilian Shield clade	9.5	9.1	9.3	7.4
Tank epiphyte clade	7.1	5.7	5.7	5.5
Puyoideae + Bromelioideae	13.2	13.4	10.1	10.0
Puy + Brom + Pitc	13.4	15.0	13.2	13.4

relatively little phylogenetic signal in slowly evolving bromeliads, so several regions must be sampled to obtain a reliable phylogenetic estimate. Finally, the pairs of plastid regions showing "significant" conflict in the ILD tests in this study are those in which one or both regions have few informative sites (Table 1). Incongruence length difference tests involving such regions are inherently unstable due to sampling error in determining the universe of characters sampled; branches supported by limited data can easily be reversed in larger data sets as the signal in individual bases is overruled by that in additional bases sampled (e.g., see Darlu and Lecointre, 2002). The fact that the apparent conflict between regions occurred only among those involving one or two regions with limited numbers of informative characters in the combined analysis, combined with the fact that such conflict should be most likely when limited numbers of characters are sampled in a phylogeny with short branches argues that the "conflict" detected by ILD tests for some pairs of regions is simply a sampling artifact and should thus be ignored.

Implications for classification—Our results confirm that the traditional division of Bromeliaceae into three subfamilies—Pitcairnioideae s.l., Tillandsioideae, and Bromelioideae (Harms, 1930), defined by possession of winged seeds, plumose seeds, and fleshy fruits, respectively—must be abandoned. Pitcairnioideae sensu Harms (1930) is paraphyletic and must be split into Brocchiniodeae, Lindmanioideae, Hechtioideae, Navioideae, Pitcairnioideae s.s., and Puyoideae to produce monophyletic subfamilies. Each of the new subfamilies is easily diagnosed based on morphology (Givnish et al., 2007), and the relationships among subfamilies found here are consistent with those demonstrated in other recent analyses (Terry et al., 1997; Crayn et al., 2000, 2004; Horres et al., 2000, 2007; Givnish et al., 2004, 2007; Barfuss et al., 2005; Schulte et al., 2005; Schulte and Zizka, 2008), but better resolved and more taxonomically inclusive.

Our results raise the question of *Puya's* monophyly. *Puya* is monophyletic but weakly supported under MP, and paraphyletic under ML and BI (Figs. 3–5). Jabaily and Sytsma (2010) found support for the monophyly of *Puya* in a combined analysis of sequences for three plastid regions (*matK, rps16, trnS-trnG*) and one single-copy nuclear gene (*PhyC*) with a far more extensive sampling of the genus. *PhyC* alone supports the monophyly of *Puya*, while the plastid data do not contradict monophyly. Given these results, *Puya's* monophyly in our MP trees, and *Puya's* possession of a striking morphological synapomorphy—e.g., petals that spiral tightly after anthesis (Smith and Downs 1974)—we consider *Puya* and Puyoideae to be monophyletic, but recognize that further tests of relationships among Chilean *Puya*, other *Puya*, and Bromelioideae would be useful. The possibility of sinking *Puya* into Bromelioideae, as suggested by Terry et al. (1997), is not appealing, given that both Bromelioideae and Puyoideae as currently defined are characterized by obvious morphological synamorphies, while the clade consisting of both subfamilies appears to lack such defining traits.

Our findings add to a growing case, developed by Schulte et al. (2005, 2009), Schulte and Zizka (2008), Zizka et al. (2009), and Jabaily and Sytsma (2010) that three small terrestrial genera from temperate Chile and the southern Andes (*Fascicularia*, *Ochagavia*, *Greigia*) are among the earliest-divergent members of subfamily Bromelioideae, together with two small terrestrial genera, wide-ranging *Bromelia* and monotypic *Deinacanthon* endemic to the semiarid Gran Chaco of southern Bolivia, Paraguay, and northern Argentina. These genera form a weakly supported clade in our ML, BI, and MP majority-rule trees, and a largely unresolved grade in our MP strict consensus tree (Figs. 3–5). All three analyses identify a further grade of small terrestrial genera sister to the remaining bromelioids, including *Pseudananas*, *Ananas*, and *Cryptanthus*; the single species of epiphytic (but nontank forming) *Acanthostachys* is closely related to *Cryptanthus.* Taxa sister to and including *Pseudananas* form the Brazilian Shield clade (61% MP bootstrap support, 81% ML bootstrap support, 100% BI bootstrap support), which arose 9.1 Ma (see *Results*). In contrast to our results, Sass and Specht (2010) recovered *Ananas* and *Araeococcus* as not being monophyletic. However, this is a result solely of those authors sampling a far greater number of species in the known "trash-can" genus *Aechmea*; almost surely, their findings will result in the errant *Aechmea* species being reclassified as members of *Ananas* or *Araeococcus.*

Almost all species in the Brazilian Shield clade—represented by the 21 species in our study, sister to and including *Aechmea drakeana-A. lingulata-Ronnbergia petersii*—form a clade of tank epiphytes endemic to the Brazilian Shield, based on the possession of tanks and the epiphytic habit by almost all these species (see Smith and Downs, 1974, 1977, 1979; Schulte et al., 2009). All three analyses support this clade, with <50% support under MP, 73% under ML, and 99% under BI (Figs. 3–5). Among these taxa, only *Araeococcus pectinatus* lacks a tank; only *Aechmea bromeliifolia*, *A. sphaerocephala*, *and Billbergia*

←

Fig. 7. Cross-verified penalized-likelihood (PL) chronogram for bromeliad evolution based on the maximum-likelihood phylogeny, using the crown and stem ages derived from the across-monocots PL analysis (see Fig. 6). Each magenta bar indicates ±1 SD around the estimated age of the corresponding node based on bootstrap resamplings.

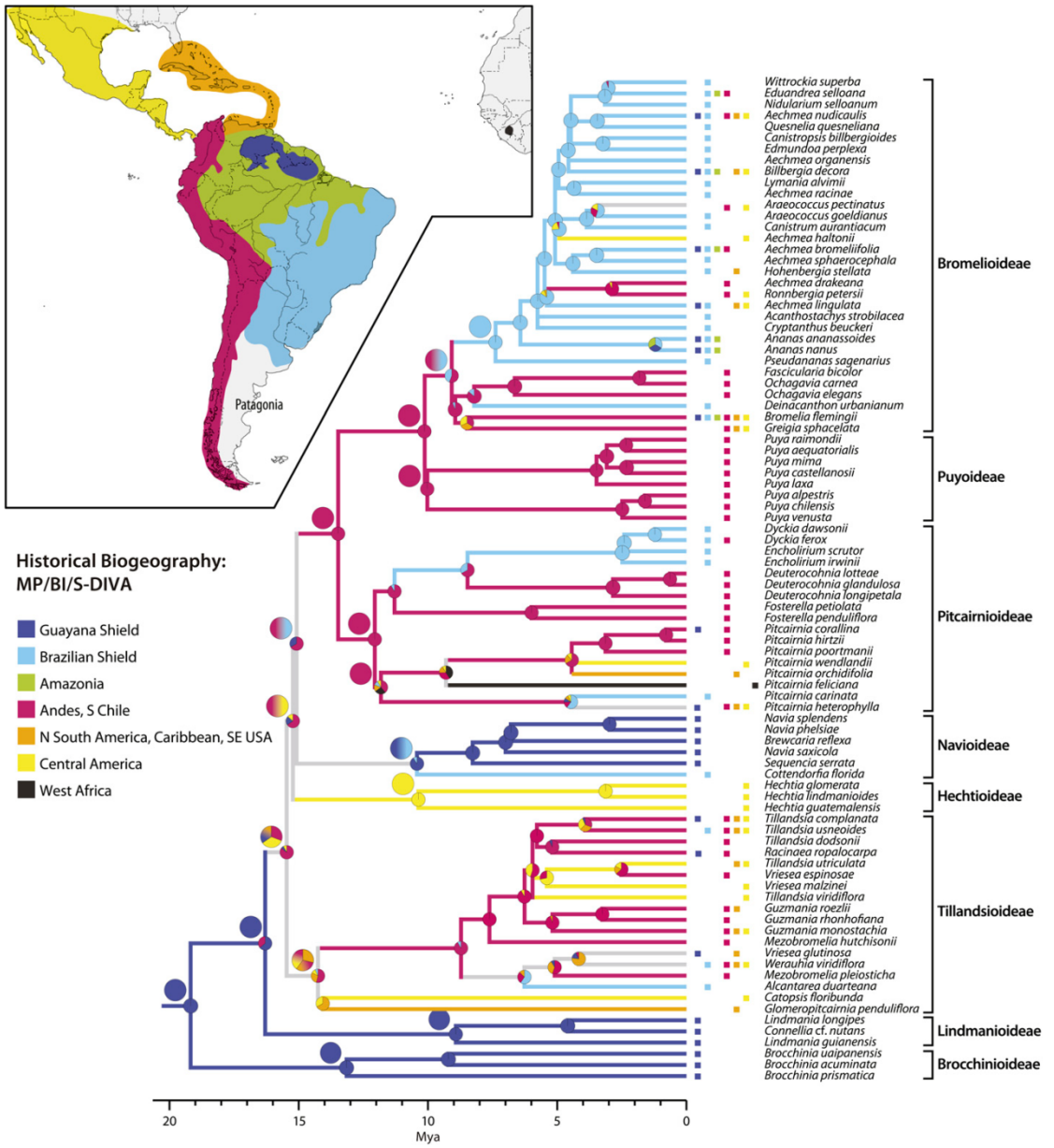

Fig. 8. Geographic evolution of Bromeliaceae calibrated against time. Present-day distribution of individual species (or of genera, in cases where wide-ranging groups are represented by one or two placeholder taxa) indicated by colored boxes. Branch colors indicate the inferred distributions of ancestral taxa under maximum parsimony (MP); gray indicates ambiguity. Pie diagrams at nodes indicate the inferred ancestral distributions under Bayesian inference (BI), with width of wedges delimited by black lines showing likelihood of alternative inferences. Larger pie diagrams displaced northwest of nodes indicate the inferred ancestral distributions under S-DIVA, with wedges delimited by black lines showing likelihood of alternative inferances, and a blend of colors within wedges signifying vicariance involving a fusion of two regions represented by those colors. Analyses involving the possible fusion of more than two areas yield similar results except for a few backbone nodes.

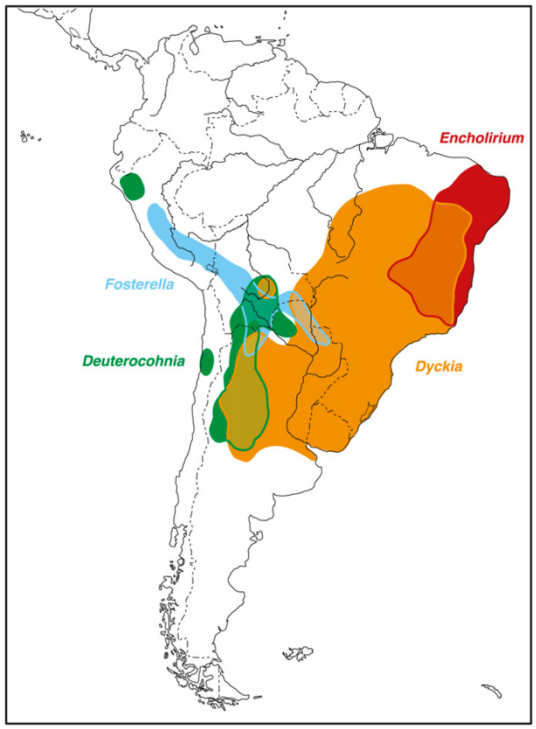

Fig. 9. Geographic distribution of genera of Pitcairnioideae minus *Pitcairnia*; the latter is broadly distributed throughout the Andes and nearby regions. Note the regional overlap of three of the four genera in the "knee" of the Andes.

decora are almost never epiphytic; and only *Aechmea drakeana*, *A. haltoni*, and *Ronnbergia petersii* are not native, at least in part, to the Brazilian Shield (Smith and Downs, 1974, 1977, 1979). Schulte et al. (2009) similarly found tanks ubiquitous (except in *Araeococcus flagellifolius*) in a clade of 28 core bromelioids sister to *Aechmea drakeana-Hohenbergia eriostachya* based on sequence data from one nuclear gene (*PRK*) and five plastid loci. That clade has a membership consistent with our bromelioid tank-epiphyte clade, but also included species of *Androlepis*, *Neoglaziovia*, *Portea*, and *Ursulaea*—four of the 12 genera not included here. Schulte et al. (2009) found that two other genera—*Orthophytum* and *Fernseea*, both species-poor terrestrial groups from the Brazilian Shield—are part of our Brazilian Shield clade. *Fernseea* is sister to all remaining elements of the Brazilian Shield clade, and *Orthophytum* is sister to *Cryptanthus*; only one species of *Fernseea* from these three genera are epiphytes or tank-formers (Schulte et al., 2009).

Our study generally agrees with Barfuss et al. (2005) on relationships within Tillandsioideae. Consistent with our ML tree, Barfuss et al. (2005) found that *Catopsis* and *Glomeropitcairnia* were sister to each other and together sister to all other tillandsioids. Also largely consistent between the two studies is the split of the remaining taxa into the tribes Vrieseeae (*Alcantarea*, *Vriesea*, *Werauhia*) and Tillandsieae (*Guzmania*, *Racinaea*, *Tillandsia*, *Viridantha*). However, in our study one species of *Vriesea* fell into Tillandsieae with 100% bootstrap support, and *Mezobromelia pleiosticha*—replacing a misidentified *Guzmania variegata* sequenced by Barfuss et al. (2005)—fell into Vrieseeae with 95% bootstrap support (Fig. 3).

Brewcaria reflexa appears to be embedded in *Navia* (Figs. 3, 4). Holst (1997) moved several species from *Navia* into *Brewcaria* based on their possessing a spicate or paniculate inflorescence, rather than the capitulate inflorescences seen in other *Navia*. This decision is not supported in the case of *Brewcaria reflexa*, the only species of that genus included in this study. Our study confirms the highly polyphyletic nature of *Aechmea,* with six independent origins indicated by our study. Sass and Specht (2010) found an even greater degree of polyphyly and paraphyly in *Aechmea* based on a much more extensive sampling of species (150) within Bromelioideae.

In a way, our findings confirm the traditional view that bromelioids and tillandsioids arose from within Pitcairnioideae s.l. (Schimper, 1888; Mez, 1904; Pittendrigh, 1948; Tomlinson, 1969; Smith and Downs, 1974; Benzing et al., 1985; Smith, 1989; Benzing, 1990). Terry et al. (1997) reached a similar conclusion, but had a different view of relationships of bromelioids to tillandsioids and the seeming isolation of *Brocchinia* because they did not sample two of our subfamilies and undersampled two others. Terry et al. (1997) also concluded that *Hechtia* was closely allied to *Dyckia*, *Encholirium*, *Abromeitiella*, and *Deuterocohnia*, rather than being a convergent lineage. Horres et al. (2000) did not exclude a close tie of *Hechtia* to xeromorphic pitcairnioids and *Puya,* but their data placed *Hechtia* in a position consistent with that found here. Givnish et al. (2007) noted that the shared possession of four to six leaf anatomical traits by *Hechtia* with *Puya* and the xeromorphic pitcairnioids as a striking instance of concerted convergence.

The classical view that bromelioids and tillandsioids emerged from within Pitcairnioideae s.l. was based not on phylogenetic analysis, but on observing that epiphytism—a highly specialized habit, with several adaptations for life on twigs and branches—is almost absent among pitcairnioids as previously circumscribed. No early writer proposed that *Brocchinia* or *Lindmania* were sister to the rest of the family, or that Pitcairnioideae s.l. were not monophyletic. Terry et al. (1997) were the first to conclude that *Brocchinia* was sister to all other bromeliads and that the traditional Pitcairnioideae were paraphyletic. That view, based on an analysis including exemplars of only 28 of 58 bromeliad genera, is confirmed and greatly amplified by the present analysis.

The remarkably long period of ca. 81 My between the rise of the bromeliads and the divergence of modern lineages from each other suggests that much extinction occurred during the intervening period, and explains the morphologically isolated position of the family and the difficulty, even with extensive molecular data sets, of identifying its sister group (see Givnish et al., 2005, 2007; Chase et al., 2006; Graham et al., 2006). Restriction of Brocchinioideae and Lindmanioideae to the Guayana Shield, the occurrence of some *Catopsis* and *Glomeropitcairnia* in or immediately adjacent to the Guayana Shield, and the near restriction to that region of Navioideae, combined with the phylogenetic relationships shown here, place the origin of Bromeliaceae in the Guayana Shield, consistent with the evidence and arguments presented by Givnish et al. (2007). The divergence of most bromelioid genera in just the last 5.5 Myr, coupled with very low rates of molecular evolution in bromeliads, explains the great difficulty investigators have had in ob-

taining a well-resolved phylogeny for bromelioids (Terry et al., 1997; Horres et al., 2000, 2007; Crayn et al., 2004; Givnish et al., 2004, 2007; Schulte et al., 2005) and the relatively limited and homoplastic morphological variation in this group (Smith and Downs, 1979; Smith and Kress, 1989, 1990; de Faria et al., 2004; Schulte and Zizka, 2008; Sass and Specht, 2010).

Historical biogeography—Our analyses show that bromeliads arose in the Guayana Shield roughly 100 Ma, spread from that hyperhumid, extremely infertile center to other parts of tropical and subtropical America starting ca. 15.4 Ma, and arrived in tropical Africa ca. 9.3 Ma. Our PL chronology implies that the extant subfamilies began to diverge from each other beginning only about 19 Ma and that invasion of drier peripheral areas in Central America (*Hechtia*) and northern South America (Tillandsioideae) began roughly 15.2 to 15.4 Ma. Brocchinioideae, Lindmanioideae, and Navioideae except *Cottendorfia* remained entirely within the Guayana Shield. The northern Andes and Central America were independently colonized by two major lineages: the core tillandsioids (*Alcantarea*, *Tillandsia*, *Vriesea*, *Werauhia*) beginning about 14.2 Ma; and *Fosterella,* beginning about 11.3 Ma. In addition, *Puya* and the early-divergent bromelioids colonized throughout the Andes, extending into temperate coastal Chile, beginning ca. 10.1 Ma (Fig. 5; all calculated ages based on stem groups). Other groups—including some *Pitcairnia* and species in several bromelioid genera (e.g., *Aechmea*, *Araeococcus*, *Neoregelia*, *Ronnbergia*)—also invaded the Andes independently, but we have not sampled enough taxa to estimate the timing and/or numbers of such events reliably. At least five additional colonizations, however, appear to be involved.

Uplift of the northern Andes beginning in the mid-Miocene, causing a shift in the course of the Amazon from a northerly route via the paleo-Orinoco toward Lake Maracaibo to an easterly course toward its present mouth (Hoorn, 1994; Hoorn et al., 1995, 2010; Potter, 1997), appears to correspond roughly to when bromeliad subfamilies began to diverge outside the Guayana Shield. This Andean uplift appears to have occurred at about the same time as the first split of modern hummingbird lineages in the Andes ca. 13 Ma, with several other Andean lineages diverging during the Pliocene and Pleistocene (Bleiweiss 1998), just as the uplift of the Columbian Andes accelerated starting ca. 3.9 Ma (Gregory-Wodzicki, 2000).

As the central and northern Andes continued to rise, they were colonized by the largely epiphytic tillandsioids between ca. 14.2 and 8.7 Ma, after that subfamily began diversifying in the northern littoral of South America, the Caribbean, and Central America. Speciation in Andean tillandsioids was explosive, resulting in ca. 1250 present-day species (Luther, 2008), more than 60 times the numbers of taxa seen in Brocchinioideae. Tillandsioids today have their great species richness in Andean Colombia, Ecuador, and Peru and range along the length of the Andes, into arid habitats on the Pacific and Caribbean littorals, and into Central America and North America north to Virginia (Smith and Downs, 1977).

How *Hechtia* colonized arid areas of Central America is unclear. The Isthmus of Panama did not close until roughly 4.4–3.1 Ma (Ibaraki, 1997; Kirby et al., 2008), so colonization from the Guayana Shield, the Caribbean or Caribbean littoral, or the Andes almost surely involved one or more bouts of long-distance seed dispersal, either directly to Central America, or via a series of arid habitats in the Lesser and Greater Antilles, or on the west slope of the Andes. Such long-distance dispersal appears plausible, given the inferred dispersal of *Fosterella* from the central Andes to dry forests in Mexico, El Salvador, and Guatemala in Central America (Rex et al., 2007), of *Greigia* and *Ochagavia* to the Juan Fernandez Islands and *Racinaea* to the Galápagos from the Andes (Smith and Downs, 1974), and of *Pitcairnia* across the tropical Atlantic to West Africa (Givnish et al., 2004, 2007). We favor direct dispersal of ancestral *Hechtia* to Central America, given the persistence of arid habitats in the Caribbean as well as coastal Peru and Chile, and the absence of *Hechtia* there. Today, *Hechtia* is restricted to arid habitats in Central America, while tillandsioids there are more broadly distributed ecologically and are especially diverse in humid montane habitats (see Smith and Downs, 1974, 1977).

Deposition of nutrient-rich Andean sediments in the Amazon basin, separating the Guayana and Brazilian Shields ecologically, accelerated ca. 11.8–11.3 Ma, corresponding to continued uplift of the northern Andes and filling of the vast Pebas wetlands of western Amazonia, as well as erosion finally cutting through the Purus Arch in central Amazonia (Figueiredo et al., 2009). Divergence of monotypic *Cottendorfia* from remaining Navioideae of the Guayana Shield about 10.4 Ma suggests that *Cottendorfia* may have arrived in the Brazilian Shield via long-distance dispersal. However, the timing of the deposition of Amazonian sediments separating the Guayana and Brazilian Shields on the Amazonian Platform is close enough in time, and the proximity of both shields close enough in space then that we should not exclude vicariance–short-distance dispersal as an alternative explanation. Three other groups also appear to have colonized the Brazilian Shield: *Dyckia-Encholirium* from the central Andes 8.5 Ma (Fig. 7); the Brazilian Shield bromelioids, most likely from the southern Andes ca. 9.1 Ma (see below); and certain species of *Bromelia*, probably from the Amazon basin, also ca. 9.1 Ma (Fig. 8). Individual species of several wide-ranging genera (e.g., *Guzmania*, *Tillandsia*, *Vriesea*) almost surely colonized the Brazilian Shield from other areas as well.

Our reconstruction suggests that Pitcairnioideae dispersed counterclockwise through time, first from the Guayana Shield to the (northern) Andes and its lowland slopes for *Pitcairnia*, then to the central Andes for the split between lineages giving rise to *Fosterella* and to the remaining genera, with a split between the puna cushion-plants of *Deuterocohnia* and arid-zone *Dyckia* in south-central Bolivia roughly 9.1 Ma, and subsequent dispersal of *Dyckia* to the Brazilian Shield and its divergence from *Encholirium* in the Horn of Brazil about 2.4 Ma (Figs. 7 and 8; see also Givnish et al., 2004, 2007).

The cradle of *Puya* appears to be Andean, but our analysis samples too few species within the genus to locate its geographic origin (see Jabaily and Sytsma, 2010). Jabaily (2009) used AFLP data to argue that *Puya* spread northward from the southern and central Andes soon after the split from the Chilean taxa. Based on our calculations, that split occurred around 10 Ma, soon after the uplift of the northern Andes began to accelerate. Divergence between Puyoideae and Bromelioideae seems likely to have occurred in and around the southern Andes, given the basal split in *Puya* between Chilean and Andean taxa, the apparent origin of *Puya* generally from the southern Andes, and the presence in the southern Andes and nearby Pacific lowlands of several members of basal grade or clade of bromelioids, including *Fascicularia*, *Greigia*, and *Ochagavia* (see Results and Schulte et al., 2005). Subsequent diversification of Bromelioideae entailed dispersal of *Bromelia* and *Ananas* throughout much of lowland South and Central America, with colonization of the Brazilian Shield independently by *Bromelia* and by the

ancestor(s) of *Fernseea* (see Schulte et al., 2005) and the large number of genera sister to it (Fig. 8). This last lineage—the Brazilian Shield clade—apparently arose 9.1 Ma (Fig. 8).

We propose that the origin of the bromelioid epiphytic clade in and around the Serra do Mar roughly 5.5 Ma corresponds to three key events, involving (1) uplift of the Serra do Mar mainly during Pliocene-Pleistocene times (Almeida, 1976; Amorim and Pires, 1996), (2) uplift of the central Andean Altiplano toward the end of the Miocene (Garzione et al., 2008), and (3) origin of a cooler, rainier climate in the Serra do Mar/Atlantic rain-forest region predicted to result from the impact of central Andean uplift on wind circulation, with increased advection of moisture from the Atlantic as winds from the Pacific were blocked (Ehlers and Poulsen, 2009). The climate models of Ehlers and Poulsen (2009) assume all other factors remained constant as the height of the Andes varied, so the actual uplift of the Serra do Mar mainly from the Pliocene to the present most likely would have caused the observed onset there of cooler, rainier, more humid conditions congenial to epiphytes starting around 5.6 Ma (Vasconcelos et al., 1992; Grazziotin et al., 2006), corresponding nearly exactly with the calculated time of origin of the bromelioid epiphytic clade. Today the Atlantic forest region, including highly diverse but largely destroyed Atlantic rain forests and cloud forests, sandy coastal restingas, mangroves, campos de altitude, and granitic outcrops of the Serra do Mar and Serra da Mantiqueira and adjacent coastal plains, are the wettest part of eastern South America, and the montane habitats are the coolest (Safford, 1999). The Serra do Mar and Serra da Mantiqueira represent the elevated southeastern rim of the tilted Brazilian Shield, and these "seas of hills" ("mares do morros") between roughly 22° and 29°S intercept heavy rainfall and fog from moisture carried by winds off the tropical south Atlantic, as well as occasional cold fronts spawned in Antarctica. Strong climatic fluctuations occurred in this montane region during the Pleistocene (e.g., Behling and Negrelle, 2001), much as they did in the northern Andes (van der Hammen, 1995).

Most bromelioids that arrived in the Brazilian Shield earlier than the origin of the epiphytic clade, during a drier phase and presumably by gradual, short-distance dispersal from the southern Andean region via a corridor of semiarid habitats, are highly xeromorphic terrestrial taxa (*Bromelia*, *Pseudananas*, *Ananas*, *Cryptanthus*, *Orthophytum*). *Fernseea*, sister to all other members of the Brazilian Shield clade (Schulte et al., 2009), is restricted to cool, moist, rocky microsites on the lofty Itatiaia Massif (2800 m a.s.l.) in the Serra da Mantiqueira (Medina et al., 2006), a mountain chain inland of the Serra do Mar in the Atlantic forest region and uplifted somewhat earlier (Amorim and Pires, 1996; Modenesi-Gauttieri and Motta de Toledo, 1996). *Fernseea* may thus have arrived directly from cool, moist habitats in the southern Andes via long-distance seed dispersal. Climatic oscillations throughout the Pleistocene included rainier phases during which the isolation of Amazonian and Atlantic rain forests from each other by semiarid vegetation may have been greatly reduced (Auler and Smart, 2001; Wang et al., 2004), which would have promoted the later dispersal of bromelioids from the Serra do Mar to other areas, and dispersal of other bromeliads (e.g., *Guzmania*, *Tillandsia*, *Vriesea*) into the Serra do Mar.

Dispersal of ancestral bromelioids from the southern Andes to the mountains of southeastern Brazil is consistent with the proposal of Schulte et al. (2005), although we envision at least two colonizations, involving a long-distance, mesic "high road" for *Fernseea* (as argued by Schulte et al., 2005) and a gradual, semiarid "low road" for the remaining taxa, with subsequent evolution of mesomorphic epiphytic taxa in the Atlantic forest region. The defining disjunction of Bromelioideae between the southern Andes and the Atlantic forest region is similar that seen in several other plant groups, including *Araucaria*, *Cordyline*, *Drimys*, *Fuchsia* sect. *Quelusia*, and *Griselinia* (Zinmeister, 1987; Berry, 1989; Katinas et al., 1999; Berry et al., 2004). Most of these cases, however, probably involved a mesic "high road" to the Brazilian Shield, either via long-distance dispersal or (more likely in these ancient groups) as relicts of more widespread mesic temperate forests in the southern hemisphere during the Tertiary. In more recently dispersed groups, gradual spread of mesic-adapted taxa from the Andes to the Brazilian Highlands during glacial cycles of the last few million years is another possibility (Safford, 1999). Although glacial/interglacial cycles had much less amplitude prior to ca. 2.8 Ma (Lisiecki and Raymo, 2005), Antarctic ice sheets are known to have advanced and retreated until at least ca. 4.9 Ma (Naish et al., 2009), so dispersal of bromelioids from the southern Andes to southeastern Brazil during a glacial period cannot be excluded.

The initial diversifications of the tillandsioid and epiphytic tank bromelioid radiations roughly 14.0–8.7 Ma and 5.5 Ma, respectively, associated with independent origins of the tank habit (Givnish et al., 2007), corresponds well with the independently derived dates of origin of diving-beetle lineages endemic to bromeliad tanks ca. 12 Ma in northern South America and ca. 4 Ma in the Serra do Mar region (Balke et al., 2008 and inferences regarding ancestral distributions). In addition, the estimated origin of *Bothrops* (fer-de-lance) species endemic to Atlantic rain forests ca. 3.8 Ma (Grazziotin et al., 2006) agrees fairly well with our estimate of the origin there of the epiphytic tank bromelioids in wet forests ca. 5.5 Ma.

Pitcairnia feliciana apparently arrived in tropical West Africa via recent long-distance dispersal from South America no earlier than about 9.3 Ma. This accords with *Maschalocephalus dinklagei* of Rapateaceae also being a product of recent long-distance dispersal, not ancient vicariance via continental drift (Givnish et al., 2000, 2004). Recent colonization might partly explain the lack of African speciation in both groups, but that seems quite unlikely; the bromelioid epiphytic clade spawned nearly 600 species in less than half the time that we estimate *Pitcairnia* and *Maschalocephalus* have been in Africa. Historical cycles of aridity (Goldblatt, 1993; Querouil et al., 2003) probably played a more important role, given that neither Rapateaceae nor *Pitcairnia* are especially drought-tolerant (Givnish et al., 2004, 2007) and that neither clade contains species with fully developed CAM photosynthesis (Crayn et al., 2001, 2004).

The African endemics of these families occupy nearly adjacent ranges: *Mascalocephalus* in savannas and forests on wet sand from Sierra Leone to Côte d'Ivoire; *Pitcairnia feliciana* on sandstone outcrops of the Fouta Djalon massif in Guinea a few hundred kilometers to the northwest (Porembski and Barthlott, 1999; Givnish et al., 2000, 2004). The Guinean Mountains maintained a wet climate during the Pleistocene, serving as a refugium for wet-climate taxa (Jahns et al., 1998; Dupont et al., 2000). Both Rapateaceae and Bromeliaceae are also likely to have been favored by infertile soils, given their origin and continued abundance in the Guayana Shield. Therefore, early vicariance of habitat—through rafting of sandstone deposits to either side of the Atlantic—followed, much later, by long-

distance dispersal appears to have caused the disjunct distributions of rapateads and bromeliads (Givnish et al., 2004). There are roughly 10 other angiosperm families with amphiatlantic distributions (Thorne, 1972, 1973); the use of fossil-calibrated molecular clocks shows that recent, long-distance dispersal probably accounts for this pattern in Melastomaceae (Renner and Meyer, 2001) and Vochysiaceae (Sytsma et al., 2004) as well, with trans-Atlantic dispersal having occurred in these families well before it did in bromeliads or rapateads.

It might be argued that, even with a sample of 90 bromeliad stratified across all subfamilies and most genera, that it would be premature to reconstruct biogeographic (or, in other contexts, morphological or ecological) ancestral character states, given that less than 3% of all extant bromeliad species are included in our analysis. We disagree. First, the full range of geographic distributions have been considered for all genera included, and less than 3% of bromeliad species have been excluded in that process. More importantly, a detailed study of biogeographic and morphological variation with Bromelioideae, based on a substantially denser sampling of taxa (150 species, ca. 17.5% of all bromelioids), showed that both groups of characters were phylogenetically highly conserved (Sass and Specht, 2010). Such conservatism supports the placeholder approach used here.

What morphological and physiological traits adapted bromeliads for life outside the Guayana Shield? How frequently did they arise? Were they acquired sequentially or nearly simultaneously? To what extent is variation among the eight bromeliad subfamilies in species number and diversification rate correlated with these traits and the environments invaded by those subfamilies? What factors make the Tillandsioideae and Bromelioideae, with 40 and 27% of all bromeliad species, respectively, especially diverse? Each of these questions will be addressed in a companion paper, building on the phylogenetic, chronological, and biogeographic reconstructions presented here and new reconstructions of the ancestral states of various morphological, physiological, and ecological characters.

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APPENDIX 1. Species, vouchers, and GenBank accessions for taxa included in this study. Taxa are grouped by subfamily within Bromeliaceae and by family outside Bromeliaceae. Sequences newly generated for this study begin with HQ or JF. Taxa for which sequences were concatenated in the combined analyses are listed sequentially with an asterisk (*). Sequences for different loci obtained from different accessions of the same species are listed after the corresponding vouchers. Missing sequences are indicated by –.

Taxon; *Voucher specimen*, Herbarium; GenBank accessions: *matK*; *ndhF*; *rps16*; *atpB-rbcL*; *psbA-trnH*; *rpl32-trnL*; *trnL* intron/*trnL-trnF* intergenic spacer

Brocchinioideae

Brocchinia acuminata L.B.Sm.; SEL 81-1937; AF162228.2; L75859; HQ913837; JF280690; HQ913663; HQ913751; HQ882715. ***Brocchinia prismatica*** L.B.Sm.; *T. Givnish s.n.*, WIS; HQ900681; AY438600; HQ913838; JF280691; HQ913664; HQ913752; HQ882716. ***Brocchinia uiapanensis*** (Maguire) Givnish; *T. Givnish 4200*, WIS; HQ900682; AY438599; HQ913839; JF280692; HQ913665; HQ913753; HQ882717.

Lindmanioideae

Connellia cf. ***nutans*** L.B.Sm.; *P. E. Berry 7741*, WIS; –; HQ895740; –; –; –; –; –. ***Lindmania guianensis*** (Beer) Mez; *W. Till 16018a*, WU; AY614019; –; AY614141; AY614385; HQ913695; –; AY614263. ***Lindmania longipes*** (L.B.Sm.) L.B.Sm.; *T. Givnish s.n.*, WIS; HQ900683; AY438605; HQ913866; JF280719; HQ913696; HQ913783; HQ882736.

Tillandsioideae

Alcantarea duarteana (L.B.Sm.) J.R.Grant; *W. Till 11052*, WU; AY614031; –; AY614153; AY614397; HQ913656; HQ913744; –; *E. Leme 2891*, HB; –; HQ895732; –; –; –; –; HQ882711. ***Catopsis floribunda*** L.B. Sm.*; MSBG 91-3; AF539963; –; –; –; –; –; ***Catopsis morreniana*** Mez*; *H.B.V. B176/80*, WU; –; HQ895739; AY614147; AY614391; HQ913669; HQ913757; HQ882721. ***Glomeropitcairnia penduliflora*** (Griseb.) Mez; *T. Givnish s.n.*, WIS; –; L75864; –; –; –; –; –; *W. Till 12012*, WU; AY614030; –; AY614152; AY614396; HQ913686; HQ913774; AY614274. ***Guzmania monostachia*** (L.) Rusby ex Mez; SEL 82-225; –; L75865; HQ913859; JF280713; HQ913688; HQ913776; HQ882732; *R. Horres H016*, FR; AY949990; –; –; –; –; –; –. ***Guzmania rhonhofiana*** Harms; SEL 80-1130; –; L75934; –; –; –; –; –; *B224/80*, WU; AY614064; –; HQ913860; AY614430; HQ913689; HQ913777; AY614308/AY614308. ***Guzmania roezlii*** (E.Morren) Mez; *H.B.V. 166/96*, WU; –; –; HQ913861; JF280714; HQ913690; HQ913778; HQ882733. ***Mezobromelia hutchisonii*** (L.B.Sm.) W.Weber & L.B.Sm.; *W. Rauh 40104*, HEID; AY614050; HQ895753; AY614172; AY614416; HQ913698; HQ913785; HQ882738. ***Mezobromelia pleiosticha*** (Griseb.) Utley & H.Luther; SEL 81-1986; AF539970; L75891; HQ913868; JF280721; HQ913699; HQ913786; HQ882739. ***Racinaea ropalocarpa*** (André) M.A.Spencer & L.B.Sm.; *B256/96*, WU; AY614083; –; AY614205; AY614449; HQ913720; HQ913807; AY61437. ***Tillandsia complanata*** Benth.; SEL 79-0519; –; L75899; –; –; –; –; –; *L. Hromadnik 2137*, WU; –; –; HQ913893; –; HQ913725; HQ913812; HQ882757; *W. Till 21085a*, WU; –; –; –; JF280746; –; –; –. ***Tillandsia dodsonii*** L.B.Sm.; *Brown 3218*, RM; –; L75879; –; –; –; –; –; *W. Rauh 34183*, WU; AY614072; –; AY614194; AY614438; HQ913726; –; –; SEL 1973-0004-033; –; –; –; –; –; HQ913813; HQ882758. ***Tillandsia usneoides*** (L.) L.; *G. Palim s.n.*, WU; AY614122; –; –; –; –; –; AY614366; *M. Barfuss s.n.*, WU; –; –; AY614243; AY614487; HQ913727; HQ913814; –; ex cult. UW-Madison greenhouses; –; HQ895767; –; –; –; –; –. ***Tillandsia utriculata*** L.; *G. Brown 3211*, RM; –; L75939; –; –; –; –; –; *W. Till 17007*, WU; AY614090; –; AY614212; AY614456; HQ913728; HQ913815; AY614334. ***Tillandsia viridiflora*** (Beer) Baker; *H.B.V. B87/80*, WU; AY614066; HQ895768; AY614188; AY614432; HQ913729; HQ913816; HQ882759. ***Vriesea espinosae*** (L.B.Sm.) Gilmartin; *G. Brown 3218*, RM; AF539978.2; –; HQ913895; JF280748; HQ913731; HQ913818; HQ882760. ***Vriesea glutinosa*** Lindl.; SEL 86-0303; –; L75914; –; –; –; –; –; *H.B.V. B444/80*, WU; GU475471; –; HQ913896; JF280749; HQ913732; HQ913819; HQ882761. ***Vriesea malzinei*** E.Morren; SEL 78-757; AF162265.2; L75915; HQ913897; JF280750; HQ913733; HQ913820; HQ882762. ***Werauhia viridiflora*** (Regel) J.R.Grant; SEL 90-0282; AF539979.2; L75910; HQ913898; JF280751; HQ913734; HQ913821; HQ882763.

Hechtioideae

Hechtia glomerata Zucc.; *M. Remmick 139*, SEL; AF162245.2; HQ895752; HQ913862; JF280715; HQ913691; HQ913779; HQ882734. ***Hechtia guatemalensis*** Mez; SEL 81-1891; –; AY438604; –; –; –; –; –; *D. Crayn s.n.*, SEL; AF162246.2; –; –; –; –; –; –; *R. Horres 088*, FR; –; –; HQ913863; JF280716; HQ913692; HQ913780; AF188821/DQ084656. ***Hechtia lindmanioides*** L.B. Sm.; *D. Crayn s.n.*, SEL; AF162247.2; –; HQ913864; JF280717; HQ913693; HQ913781; HQ882735.

Navioideae

Brewcaria reflexa (L.B.Sm.) B.Holst; Givnish et al., 1997; HQ900680; –; HQ913836; JF280689; HQ913662; HQ913750; HQ882714. ***Cottendorfia florida*** Schult. & Schult.f.; SEL 96-0695; –; AY438602; –; –; –; –; –; *E. Leme 3692*, HB; AF162230.2; –; –; –; –; –; –; *T. Givnish s.n.*, WIS; –; –; HQ913843; JF280697; HQ913671; HQ913759; HQ882722. ***Navia phelpsiae*** L.B.Sm.; MSBG 1986-0523A; AF162249.2; HQ895754; HQ913869; JF280722; HQ913700; HQ913787; HQ882740. ***Navia saxicola*** L.B.Sm.; *T. Givnish s.n.*, WIS; HQ900684; AY208983; HQ913870; JF280723; HQ913701; HQ913788; HQ882741. ***Navia splendens*** L.B.Sm.; SEL 83-0288; –; L75892; –; –; –; –; –; *R. Horres 034*, FR; GU475468; –; HQ913871; JF280724; HQ913702; HQ913789; HQ882767. ***Sequencia serrata*** (L.B.Sm.) Givnish; *T. Givnish s.n.*, WIS; HQ900688; AY438601; HQ913891; JF280744; HQ913723; HQ913810; HQ882756.

Pitcairnioideae

Deuterocohnia glandulosa E.Gross; *L. Hromadnik 5167*, HEID; EU681893; –; –; –; –; –; –; *R. Horres 090*, FR; –; HQ895742; HQ913846; JF280700; HQ913674; HQ913762; AF188784/DQ084652. ***Deuterocohnia longipetala*** (Baker) Mez; *Marnier-Lapostelle s.n.*; –; AY208984; –; –; –; –; MSBG 075767; AF162231.2; –; HQ913847; JF280701; HQ913675; HQ913763; HQ882724. ***Deuterocohnia lotteae*** (Rauh) M.A.Spencer & L.B.Sm.; MSBG 94-142; AF162232.2; –; –; –; –; –; –; *R. Horres 084*, FR; –; HQ895743; HQ913848; JF280702; HQ913676; HQ913764; AF188783/DQ084566. ***Dyckia dawsonii*** L.B.Sm.; MSBG 1994-0146A; AF162234.2; HQ895744; HQ913849; JF280703; HQ913677; HQ913765; HQ882725. ***Dyckia ferox*** Mez; MSBG 1996-0211A; AF162235.2; HQ895745; HQ913850; JF280704; HQ913678; HQ913766; HQ882726. ***Encholirium irwinii*** L.B.Sm.; *E. Leme 2881*, HB; AF162237.2; HQ895748; HQ913854; JF280708; HQ913682; HQ913770; HQ882729. ***Encholirium scutor*** (L.B.Smith) Rauh; MSBG 1995-0113A; AF162239.2; HQ895747; HQ913853; JF280707; HQ913681; HQ913769; HQ882728. ***Fosterella penduliflora*** (C.H.Wright) L.B.Sm.; SEL 69-1976-12; –; L75863; –; –; –; –; –; *R. Horres 086*, FR; AY949996; –; HQ913856; JF280710; HQ913684; HQ913772; AF188782/DQ084571. ***Fosterella petiolata*** (Mez) L.B.Sm.; MSBG 1995-0007A; AF162242.2; HQ895750; HQ913857; JF280711; HQ913685; HQ913773; HQ882731. ***Pitcairnia carinata*** Mez; *G. Brown 3173*, RM; AF539974.2; L75902; HQ913875; JF280728; HQ913706; HQ913793; HQ882745. ***Pitcairinia corallina***

Linden & André; SEL 86-0574; AF162252; AY438608; –; –; –; –; –; *R. Horres 094*, FR; –; –; HQ913876; JF280729; HQ913707; HQ913794; HQ882768. ***Pitcairinia feliciana*** (A.Chev.) Harms & Mildbr.; SEL 98-0116; –; AY438609; –; –; –; –; –; *T. Givnish s.n.*, WIS; HQ900685; –; HQ913877; JF280730; HQ913708; HQ913795; HQ882746. ***Pitcairnia heterophylla*** (Lindl.) Beer; *R. Horres 2024*, FR; AF162254.2; HQ895757; HQ913878; JF280731; HQ913709; HQ913796; AF188789/DQ084649. ***Pitcairnia hirtzii*** H. Luther; SEL 93-294; AF539972; L75901; HQ913879; JF280732; HQ913710; HQ913797; HQ882747. ***Pitcairnia orchidifolia*** Mez; MSBG 1994-0036A; AF162255.2; –; HQ913880; JF280733; HQ913711; HQ913798; HQ882748. ***Pitcairnia poortmanii*** André; MSBG 1991-0018A; AF539975.1; –; HQ913881; JF280734; HQ913712; HQ913799; HQ882749. ***Pitcairnia wendlandii*** Baker; MSBG 1996-0529A; AF539976.1; HQ895758; HQ913882; JF280735; HQ913713; HQ913800; HQ882750.

Puyoideae

Puya aequatorialis André; SEL 93-211; AF162260.2; L75903; HQ913884; JF280737; HQ913715; HQ913802; HQ882752. ***Puya alpestris*** (Poepp.) Gay; *R. S. Jabaily 177*, WIS; –; HQ895760; JF280754; JF280764; –; JF280758; JF29926; *R. Horres 060*, FR; AY949998; –; –; –; –; –; –. ***Puya castellanosii*** L.B.Sm.; *R. S. Jabaily 149*, WIS; FJ968190; HQ895761; JF280755; –; JF280762; JF280759; JF299261. ***Puya chilensis*** Molina; *R. S. Jabaily 164*, WIS; HQ900686; –; –; –; –; –; –; *T. Givnish s.n.*, WIS; –; HQ895762; HQ913885; JF280738; HQ913716; HQ913803; HQ882753. ***Puya laxa*** L.B.Sm.; Crayn et al., 2004; AF162262; –; –; –; –; –; –; *R. Horres 006*, FRP; –; HQ895763; HQ913886; JF280739; HQ913717; HQ913804; AF188794/DQ084563. ***Puya mima*** L.B.Sm. & Read; *R. S. Jabaily 228*, WIS; FJ968231; HQ895764; JF280756; JF280765; JF280763; JF280760; JF299262. ***Puya raimondii*** Harms; *T. Givnish s.n.*, WIS; HQ900687; AY438611; HQ913887; JF280740; HQ913718; HQ913805; HQ882754. ***Puya venusta*** (Baker) Phil.; *R. S. Jabaily 166*, WIS; FJ968194; HQ895765; JF280757; –; –; JF280761; JF299263.

Bromelioideae

Acanthostachys strobilacea (Schult. & Schult.f.) Klotzsch; *R. Horres 019*, FR; AY950021; HQ895726; HQ913823; JF280677; HQ913648; HQ913736; AF188765/DQ084606. ***Aechmea bromeliifolia*** (Rudge) Baker; *K. Schulte 051202-4*, FR; GU475466; HQ895727; HQ913824; JF280678; HQ913649; HQ913737; HQ882707. ***Aechmea drakeana*** André; *G. Zizka 1100*, FRP; AY950043; HQ895728; HQ913825; JF280679; HQ913650; HQ913738; AF188772/DQ084588. ***Aechmea haltonii*** H.Luther; SEL 85-1447; AF539960.2; L75844; HQ913826; JF280680; HQ913651; HQ913739; HQ882708. ***Aechmea lingulata*** (L.) Baker; *Faria 81*, RFA; JF295091; HQ895729; –; –; –; –; –; *K. Schulte 101203-1*, FR; –; –; HQ913827; JF280681; HQ913652; HQ913740; HQ882709. ***Aechmea nudicaulis*** (L.) Griseb.; *K. Schulte 200603-1*, FR; –; –; HQ913828; –; HQ913653; –; DQ084689/DQ084589; *W. Till 18094*, WU; AY614024; –; –; AY614390; –; HQ913741; –. ***Aechmea organensis*** Wawra; *Wendt 342*, RFA; JF295090; HQ895730; –; –; –; –; –; *K. Schulte 250205-1*, FR; –; –; HQ913829; JF280682; HQ913654; HQ913742; HQ882710. ***Aechmea racinae*** L.B.Sm.; *Faria 80*, RFA; JF295089; HQ895731; –; –; –; –; –; *K. Schulte 120203-1*, FR; –; –; HQ913830; JF280683; HQ913655; HQ913743; DQ084691/DQ084583. ***Aechmea sphaerocephala*** Baker; *R. Horres 030b*, FR; AY950045; –; HQ913842; JF280696; HQ913670; HQ913758; AF188770/DQ084578. ***Ananas ananassoides*** (Baker) L.B.Sm.; *G. Brown 3129*, RM; AF162227.2; L75845; HQ913831; JF280684; HQ913657; HQ913745; HQ882712. ***Ananas nanus*** (L.B.Sm.) L.B.Sm.; *R. Horres & K. Schulte 050401-9*, FR; AY950054; –; HQ913832; JF280685; HQ913658; HQ913746; DQ084695/DQ084573; SEL 1991-0469; –; HQ895733; –; –; –; –. ***Araeococcus goeldianus*** L.B.Sm.; *Moonen s.n.*, SEL; –; HQ895734; –; –; –; –; –; *K. Schulte 100203-1*, FR; AY950002; –; HQ913833; JF280686; HQ913659; HQ913747; DQ084630/DQ084697. ***Araeococcus pectinatus*** L.B. Sm.; SEL 85-231; AF539961.2; L75846; HQ913834; JF280687; HQ913660; HQ913748; HQ882713. ***Billbergia decora*** Poepp. & Endl.; *R. Horres 129*, FR; AY950050; HQ895735; HQ913835; JF280688; HQ913661; HQ913749; DQ084698/DQ084624. ***Bromelia chrysantha*** Jacq.*; MSBG 1983-0286A; AF539962; –; JF280753; –; –; –; –. ***Bromelia flemingii*** I.Ramirez & Carnevali*; SEL 1997-0231; –; HQ895736; –; JF280693; HQ913666; HQ913754; HQ882718. ***Canistropsis billbergioides*** (Schult. & Schult.f.) Leme; *E. Leme 171*, RFA; JF295092; HQ895737; –; –; –; –; –; *K. Schulte 061202-1*, FR; –; –; HQ913840; JF280694; HQ913667; HQ913755; HQ882719. ***Canistrum aurantiacum*** E.Morren; *E. Leme 567*, RFA ; JF295094; HQ895738; –; –; –; –; –; K. Schulte 300508-4, FR; –; –; HQ913841; JF280695; HQ913668; HQ913756; HQ882720. ***Cryptanthus beuckeri*** E.Morren; SEL 89-499; AF539965.2; L75856; HQ913844; JF280698; HQ913672; HQ913760; HQ882723. ***Deinacanthon urbanianum*** (Mez) Mez; *R. Horres H018*, FRP; AY950017; HQ895741; HQ913845; JF280699; HQ913673; HQ913761; AF188781/DQ084607. ***Edmundoa perplexa*** (L.B.Sm.) Leme; MSBG 1987-264; AF539967.2; HQ895746; HQ913851; JF280705; HQ913679; HQ913767; HQ882727. ***Eduandrea selloana*** (Baker) Leme, W.Till, G.K.Br., J.R.Grant & Govaerts; *E. Leme 1830*, HB; JF295093; L75894; –; –; –; –; –; *H.B.V. B00B95-1*, WU; –; –; HQ913852; JF280706; HQ913680; HQ913768; HQ882743. ***Fascicularia bicolor*** (Ruiz & Pav.) Mez; *G. Zizka 1790*, FRP; AY950023; –; –; –; –; –; –; *D. Vandervoort s.n.*, WU; –; HQ895749; HQ913855; JF280709; HQ913683; HQ913771; HQ882730. ***Greigia sphacelata*** (Ruiz & Pav.) Regel; *K. Schulte 230305-4*, FR; AY950015; HQ895751; HQ913858; JF280712; HQ913687; HQ913775; AF188779/DQ084599. ***Hohenbergia stellata*** Schult. & Schult.f.; *R. Horres 037*, FRP; AY950026; –; HQ913865; JF280718; HQ913694; HQ913782; AF188774/DQ084609. ***Lymania alvimii*** (L.B.Sm. & Read) Read; SEL 90-297; –; L75907; HQ913867; JF280720; HQ913697; HQ913784; HQ882737; *R. Horres & K. Schulte 050401-4*, FR; AY950000; –; –; –; –; –; –. ***Neoregelia pineliana*** (Lem.) L.B.Sm.; SEL 86-261; AF539971; L75893; –; –; –; –; –; *R. Horres & K. Schulte 210601-1*, FR; –; –; HQ913872; JF280725; HQ913703; HQ913790; HQ882742. ***Ochagavia carnea*** (Beer) L.B.Sm. & Looser; *R. Horres 117*, FR; –; HQ895755; HQ913873; JF280726; HQ913704; HQ913791; HQ882744; *R. Horres 115*, FR; EU681905; –; –; –; –; –; –. ***Ochagavia elegans*** Phil.; *R. Horres 23a*, FR ; AY950006; HQ895756; HQ913874; JF280727; HQ913705; HQ913792; AF188778/DQ084603. ***Pseudananas sagenarius*** (Arruda) Camargo; *M. W. Chase 24447*, K; GU475470; HQ895759; HQ913883; JF280736; HQ913714; HQ913801; HQ882751. ***Quesnelia quesneliana*** (Brongn.) L.B.Sm.; *K. Schulte 300508-6*, FR; –; –; HQ913888; JF280741; HQ913719; HQ913806; HQ882755; *Wendt 335*, RFA; JF295095; HQ895766; –; –; –; –; –. ***Ronnbergia petersii*** L.B.Sm.; SEL 78-907; –; L75897; –; –; –; –; –; *K. Schulte 170203-5*, FR; AY950001; –; HQ913890; JF280743; HQ913722; HQ913809; DQ084718/DQ084632. ***Wittrockia superba*** Lindm.; *R. Horres & K. Schulte 050401-8*, FR; AY950025; HQ895769; HQ913899; JF280752; HQ913735; HQ913822; AF188767/DQ084611.

Rapateaceae

Rapatea paludosa Aubl.; *K. J. Sytsma et al. 5157*, WIS; –; AF207623; HQ913889; JF280742; HQ913721; HQ913808; HQ882764.

Sparganiaceae

Sparganium sp.; *T. Givnish s.n.*, WIS; AB088802; AY191213; HQ913892; JF280745; HQ913724; HQ913811; HQ882765.

Typhaceae

Typha angustifolia L.*; *Graham 1040*, TRT; –; U79230; –; –; –; –; –. ***Typha latifolia*** L.*; *T. Givnish s.n.*, WIS; DQ069587; –; HQ913894; JF280747; HQ913730; HQ913817; HQ882766.

Part 2

Optimization of nuclear DNA markers and their application in phylogenetic reconstructions of subfamilies Bromelioideae and Tillandsioideae (Bromeliaceae)

Chapter 2

Phylogeny of Bromelioideae (Bromeliaceae) inferred from nuclear and plastid DNA loci reveals the evolution of the tank habit within the subfamily

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Status: published, *Molecular Phylogenetics and Evolution* 51 (2): 327–339. 2009.

Contribution: primer design, data collection, preliminary data analyses, manuscript writing/editing.

Molecular Phylogenetics and Evolution 51 (2009) 327–339

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Phylogeny of Bromelioideae (Bromeliaceae) inferred from nuclear and plastid DNA loci reveals the evolution of the tank habit within the subfamily

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ARTICLE INFO

Article history:
Received 19 August 2008
Revised 12 February 2009
Accepted 12 February 2009
Available online 21 February 2009

Keywords:
Bromelioideae
Low-copy nuclear gene
Phosphoribulokinase
PRK
*atp*B-*rbc*L spacer
*trn*L-*trn*F spacer
*trn*L intron
*mat*K
Character evolution
Sepal symmetry
Tank habit

ABSTRACT

Phylogenetic relationships within subfamily Bromelioideae (Bromeliaceae, Poales) were inferred using DNA sequence data from the low-copy nuclear gene phosphoribulokinase (PRK) and five plastid loci (*mat*K gene, 3′*trn*K intron, *trn*L intron, *trn*L-*trn*F spacer, *atp*B-*rbc*L spacer). The PRK dataset exhibited a considerably higher proportion of potentially informative characters than the plastid dataset (16.9% vs. 3.1%), leading to a higher resolution and improved nodal support of the resulting phylogenies. *Bromelia* is resolved as sister to the remainder of the subfamily, albeit this relationship receives only weak nodal support. The basal position of *Bromelia*, as well as *Deinacanthon*, *Greigia*, *Ochagavia*, *Fascicularia* and *Fernseea* within the subfamily is corroborated and the remainder of the subfamily forms a highly supported clade (the eu-bromelioids). By the inclusion of nuclear data the sister group position of *Fernseea* to the eu-bromelioids is now highly supported. Within the eu-bromelioids the resolution of the clade representing the more advanced core bromelioids has increased and further demonstrates the highly problematic generic concept of *Aechmea* as well as *Quesnelia*.

Moreover, the data were used to examine the evolution of sepal symmetry and the tank habit. Tracing of character transitions onto the molecular phylogeny implies that both characters have undergone only few transitions within the subfamily and thus are not as homoplasious as previously assumed. The character state reconstruction reveals the great importance of the evolution of the tank habit for the diversification of the core bromelioids.

1. Introduction

Repeated invasion of related lineages of organisms into similar environments, as for example those characterized by extreme drought and nutrient deficiency, can result in parallel as well as convergent evolution. The multiple and independent origin of similar features within closely related lineages is one of the important challenges in taxonomy, as it renders the recognition of phylogenetic relationships among those lineages difficult. In Bromelioideae similar abiotic and biotic pressures of the colonized habitats in the different regions of tropical and subtropical America have been postulated as one possible reason for the considerable degree of homoplasies displayed by the subfamily, as for example in inflorescence and flower morphology (Faria et al., 2004; Schulte and Zizka, 2008).

The Bromelioideae, with 32 genera and approx. 800 species (Smith and Till, 1998; Luther 2006), are one of eight subfamilies currently recognized within Bromeliaceae (Poales) (Givnish et al. 2007) and display striking ecological versatility, occupying a wide range of terrestrial, lithophytic and epiphytic habitats. They are distributed throughout tropical and subtropical America, with a centre of diversity in southeastern Brazil, particularly the Atlantic rain forest (Smith and Downs, 1979). Whereas the monophyly of Bromelioideae is strongly supported by both, morphological and molecular data (e.g. Terry et al., 1997; Crayn et al., 2004; Givnish et al., 2004, 2007; Schulte et al., 2005), the inter- and infrageneric relationships of the subfamily are the most poorly understood within the family (Benzing, 2000; Brown and Leme, 2000). The generic delimitation within the subfamily is regarded as especially problematic because morphological characters often prove to be homoplastic and hence fail to delimit natural groups and because several genera are defined by unique combinations of characters, rather than by traditional synapomorphies. The arising problems are especially evident in the *Aechmea* alliance (e.g. Faria et al., 2004; Horres et al., 2007; de Oliveira et al., 2007; Schulte and Zizka, 2008). Furthermore, potentially useful characters are often inaccessible in herbarium material and thus their variability is hardly understood. Frequent changes of generic limits within Bromelioideae reflect the considerable uncertainties concerning the taxo-

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doi:10.1016/j.ympev.2009.02.003

nomic value of morphological characters (e.g. Smith and Kress, 1989, 1990; Read and Baensch, 1994; Brown and Leme, 2005; Betancur and Salinas, 2006; de Sousa and Wendt, 2008). Furthermore, since the last comprehensive monograph of the subfamily (Smith and Downs, 1979) the number of described species has increased by more than one third (Luther, 2006), and a modern taxonomic revision is urgently needed.

Several molecular studies employing different plastid markers have dealt with the phylogenetic relationships of Bromelioideae (e.g. Terry et al., 1997; Horres et al., 2000, 2007; Givnish et al., 2004, 2007; Barfuss et al. 2005; Schulte et al., 2005; Schulte and Zizka, 2008). They consistently resolved *Puya* (Pitcairnioideae *s.l.*), a genus of terrestrial plants with a principally Andean distribution, as sister group to the Bromelioideae.

Due to an extraordinary low amount of sequence divergence within plastid DNA regions in Bromelioideae in general (Horres et al., 2000, 2007; Crayn et al., 2004; Schulte et al., 2005, Schulte and Zizka, 2008), resolution among bromelioid genera based on only one or two plastid regions has remained poor (e.g. Horres et al., 2000, 2007; Crayn et al., 2004). By the combination of several plastid regions, resolution was increased and several genera of putatively basal position (*Bromelia*, *Deinacanthon*, *Greigia*, *Ochagavia/Fascicularia* and *Fernseea*) were identified, but without resolving relationships between those genera. The remaining Bromelioideae formed a highly supported group with *Fernseea* as putative sister group, but this relationship received only weak bootstrap support. Among the former, the majority of genera (e.g. *Aechmea*, *Billbergia*, *Neoregelia*, *Nidularium*) formed a poorly resolved clade, representing the core bromelioids (Schulte et al., 2005; Schulte and Zizka, 2008).

Within the family a progression towards an increasing independence from water and nutrition supply from the soil is found in different evolutionary lines (e.g. Tietze, 1906; Pittendrigh, 1948; Benzing, 2000). Unique trichomes facilitating water uptake via the leaf surface, a tank habit allowing for water and nutrient capture in external reservoirs formed by the leaf sheaths, and the crassulacean acid metabolism (CAM), which diminishes water loss during photosynthesis, are regarded as key innovations that allow for the successful colonization of xeric and nutrition deficient environments by the family (Tietze, 1906; Pittendrigh, 1948; Medina, 1974; Crayn et al., 2004; Givnish et al., 1997; Givnish et al., 2007). Within the family different eco-morphological types can be discerned reflecting an increasing independence from the substrate (Tietze, 1906; Pittendrigh, 1948; Benzing, 2000) on the basis of the characteristics regarded as mainly responsible for this development (e.g. increasing differentiation of leaf trichomes, increasing importance of water uptake via leaf trichomes, formation of external water reservoirs, reduction of root system, photosynthetic mode: C3/CAM).

In all of the concepts the more primitive types are characterized by a well developed root system, reliance on water and nutrition uptake via soil roots and little or no external water storage capacity (every leaf sheath forms a distinct phytotelm). In the more advanced types the leaf trichomes become more important for water and nutrition uptake. The development of a central tank, formed by the leaf sheaths of the rosulate plant is seen as an important progression. The lack of a central tank and the complete reliance on the indumentum for the water and nutrient supply characterizes the most advanced type realized within the extreme atmospheric Tillandsioideae. Due to the poor phylogenetic resolution within Bromelioideae the lines of evolution of the eco-morphological types within the subfamily have remained unclear. The last comprehensive systematic treatment of Smith and Downs (1979) implies a high evolutionary lability of these types within the subfamily.

The lack of phylogenetically informative markers and the sole reliance on information from the plastid genome have been up to now principal weaknesses of phylogenetic research in Bromeliaceae. Independent attempts by a number of workers to use the nuclear ribosomal DNA-region ITS (internal transcribed spacer) have failed due to amplification difficulty and insufficient phylogenetic variability (Barfuss, unpublished data).

Low-copy nuclear loci provide some of the most variable and phylogenetically informative molecular markers available. They are especially advantageous in obtaining resolution among rapidly diversifying lineages or at low taxonomic levels, particularly where universal markers such as plastid and nuclear ribosomal DNA fail to resolve relationships due to low sequence variability (Sang, 2002; Mort and Crawford, 2004; Small et al., 2004). However, their scarce use in phylogenetic analysis is due to practical and theoretical complications, e.g. in obtaining the target regions, the differentiation between paralogous and orthologous regions and the presence of heterozygosity, which requires cloning (Sang, 2002; Mort and Crawford, 2004; Small et al., 2004). Low-copy nuclear regions have been employed with considerable success at a range of taxonomic levels, for example in Arecaceae (Lewis and Doyle, 2001, 2002; Norup et al., 2006; Gunn, 2004; Roncal et al., 2005; Thomas et al., 2006; Loo et al., 2006).

PRK is a low-copy nuclear gene that encodes phosphoribulokinase, a key regulatory enzyme of the Calvin cycle for photosynthetic carbon dioxide assimilation. Thus far its use in phylogenetic research has been restricted to the monocot family Arecaceae, where it was useful to resolve relationships at generic as well as species level (e.g. Loo et al., 2006; Norup et al., 2006; Thomas et al., 2006). Based on the promising results of these studies, PRK was considered potentially useful in the reconstruction of relationships within the Bromelioideae.

The goals of this study were (a) to explore the phylogenetic utility of PRK in resolving relationships within Bromelioideae, (b) to elucidate the intergeneric relationships within the subfamily by combining plastid and nuclear genetic markers, and (c) to examine character transformation patterns in key morphological features to discuss character evolution within Bromelioideae.

2. Materials and methods

2.1. Taxon sampling

In total, DNA sequences of 48 species from 24 genera were analyzed in the present study. Of subfamily Bromelioideae 43 species from 23 genera were sampled, representing all principal lineages within the subfamily according to previous molecular studies (Schulte et al., 2005; Schulte and Zizka, 2008). Within *Aechmea* 13 species were studied representing all seven subgenera (*Aechmea*, *Lamprococcus* (Beer) Baker, *Macrochordion* (de Vriese) Baker, *Ortgiesia* (Regel) Mez, *Platyaechmea* (Baker) Baker, *Podaechmea* Mez, and *Pothuava* (Baker) Baker) recognized by Smith and Till (1998). Five representatives of the genus *Puya* (Pitcairnioideae *s.l.*), consistently revealed as sister group of Bromelioideae by molecular studies (e.g. Givnish et al., 2004, 2007; Crayn et al., 2004; Schulte et al., 2005, Schulte and Zizka, 2008), were chosen as outgroup.

Sequences from one low-copy nuclear gene (phosphoribulokinase, PRK) and five plastid regions (*atp*B-*rbc*L spacer, *trn*L intron, *trn*L-*trn*F spacer, *mat*K gene, and part of the adjacent 3′*trn*K intron) were analyzed. The PRK sequence data were generated specifically for this study, and combined with cpDNA sequence data largely taken from our previous studies (see Table 1). Plant material was derived from the Palmengarten Frankfurt/Main, the Botanical Gardens of the Universities Heidelberg, Berlin-Dahlem, Kassel and from the Royal Botanic Gardens, Kew. Vouchers are deposited in one of the following herbaria: B, FR, FRP, HEID, K. Information on

Table 1
Studied material. References: Ref. 1: this study; Ref. 2: Schulte and Zizka (2008); Ref. 3: Horres et al. (2000); Ref. 4: Horres et al. (2007); Ref. 5: Schulte et al. (2005). *Abbreviations:* B, Herbarium Berlin-Dahlem; BGB, Botanical Garden Berlin-Dahlem; GHB, Herbarium of the Botanical Garden Berlin-Dahlem; HEID, Botanical Garden and Herbarium of the University of Heidelberg; FR, Herbarium Senckenbergianum; FRP, Botanical Garden and Herbarium Palmengarten, Frankfurt; K, Herbarium of the Royal Botanic Gardens, Kew; KEW: Royal Botanic Gardens Kew.

Species	Accession no. living collection/ herbarium specimen	DNA-isolate no.	GenBank No./reference-No.					
			PRK	Clone (PRK)	*atp*B-*rbc*L spacer	*trn*L intron	*trn*L-*trn*F spacer	*mat*K, 3′*trn*K
Bromelioideae								
Acanthostachys strobilacea (Schult.f.) Klotzsch	FRP 98-16986-0/Horres 019 (FR)	H 019	EU780812/ Ref. 1	K4	EU219694/ Ref. 2	AF188765/ Ref. 3	DQ084606/ Ref. 4	AY950021/ Ref. 5
Aechmea calyculata E.Morren ex Baker	HEID 103296/Schulte 240203-9 (FR)	H 184			EU219713/ Ref. 2	DQ084674/ Ref. 4	DQ084593/ Ref. 4	AY950040/ Ref. 5
Aechmea calyculata E.Morren ex Baker	HEID 103296/Schulte 270404-6 (FR)	J 034	EU780818/ Ref. 1	K5				
Aechmea distichantha Lem.	FRP 88-16753-2/Zizka 1549 (FRP), Horres 008 (FR)	H 008	EU780823/ Ref. 1	K2	EU219714/ Ref. 2	DQ084643/ Ref. 3	DQ084579/ Ref. 4	AY950041/ Ref. 5
Aechmea drakeana André	FRP 98-16955-2/ Zizka 1100 (FRP)	H 042	EU780814/ Ref. 1	K8	EU219716/ Ref. 2	AF188772/ Ref. 3	DQ084588/ Ref. 4	AY950043/ Ref. 5
Aechmea farinosa (Regel) L.B.Sm.	FRP 98-16961-3/Zizka 1108 (FRP)	H 272	EU780820/ Ref. 1	K7	EU219704/ Ref. 2	DQ084677/ Ref. 4	DQ084586/ Ref. 4	AY950031/ Ref. 5
Aechmea filicaulis (Griseb.) Mez	FRP 98-16863-0/Horres & Schulte 180701-6 (FR)	H 248	EU780822/ Ref. 1	K1	EU219709/ Ref. 2	DQ084679/ Ref. 4	DQ084576/ Ref. 4	AY950036/ Ref. 5
Aechmea gracilis Lindm.	FRP 98-16949-3/Schulte 280203-1 (FR)	H 043	EU780816/ Ref. 1	K4	EU219711/ Ref. 2	DQ084682/ Ref. 4	DQ084594/ Ref. 4	AY950038/ Ref. 5
Aechmea kertesziae Reitz	FRP 98-16935-3/Zizka 1177 (FRP)	H 270	EU780817/ Ref. 1	K4	EU219712/ Ref. 2	DQ084683/ Ref. 4	DQ084595/ Ref. 4	AY950039/ Ref. 5
Aechmea lamarchei Mez	BG Berlin-Dahlem 118-37-74-86/11309 (GHB)	H 242	EU780824/ Ref. 1	K2	EU219717/ Ref. 2	DQ084684/ Ref. 4	DQ084590/ Ref. 4	AY950044/ Ref. 5
Aechmea lueddemanniana (K.Koch) Mez	FRP 95-14215-0/Schulte 100203-3 (FR); Schulte 010305-1 (FR)	H 150	EU780827/ Ref. 1	K1	EU219702/ Ref. 2	DQ084685/ Ref. 4	DQ084596/ Ref. 4	AY950029/ Ref. 5
Aechmea mertensii (G.Mey.) Schult.f.	FRP 98-16873-0/Zizka 1572 (FRP)	H 044	EU780821/ Ref. 1	K6	EU219708/ Ref. 2	DQ084686/ Ref. 4	DQ084575/ Ref. 4	AY950035/ Ref. 5
Aechmea mexicana Baker	HEID 104025/Schulte 240203-12 (FR); Schulte 171103-25 (FR)	H 256	EU780826/ Ref. 1		EU219701/ Ref. 2	DQ084688/ Ref. 4	DQ084597/ Ref. 4	AY950028/ Ref. 5
Aechmea pimenti-velosoi Reitz	FRP 98-16878-0/Schulte 230305-1 (FR)	J 013	EU780815/ Ref. 1	K1	EU780879/ Ref. 1	EU780855/ Ref. 1	EU780867/ Ref. 1	EU780843/ Ref. 1
Aechmea racinae L.B.Sm.	FRP 98-16934-3/Schulte 120203-1 (FR)	H 257	EU780819/ Ref. 1	K1	EU219703/ Ref. 2	DQ084691/ Ref. 4	DQ084583/ Ref. 4	AY950030/ Ref. 5
Ananas nanus (L.B.Sm.) L.B.Sm.	FRP s.n./Horres & Schulte 050401-9 (FR)	H 040	EU780810/ Ref. 1	K1	EU219727/ Ref. 2	DQ084695/ Ref. 4	DQ084573/ Ref. 4	AY950054/ Ref. 5
Ananas fritzmuelleri Carmago	KEW 1972-9/Chase 23823 (K)	KEW 23823	EU780811/ Ref. 1		EU780880/ Ref. 1	EU780856/ Ref. 1	EU780868/ Ref. 1	EU780844/ Ref. 1
Androlepis skinneri (K.Koch) Brongn. ex Houllet	FRP 97-16793-2/Schulte 140105-12 (FR)	H 048	EU780829/ Ref. 1	K3	EU219678/ Ref. 2	AF188780/ Ref. 3	DQ084610/ Ref. 4	AY950005/ Ref. 5
Araeococcus flagellifolius Harms	KAS s. n./Rex 260105-1 (FR)	K 9	EU780834/ Ref. 1	K1	EU219676/ Ref. 2	DQ084696/ Ref. 4	DQ084629/ Ref. 4	AY950003/ Ref. 5
Araeococcus goeldianus L.B.Sm.	FRP 99-18256-2/Schulte 100203-1 (FR)	H 206	EU780833/ Ref. 1		EU219675/ Ref. 2	DQ084697/ Ref. 4	DQ084630/ Ref. 4	AY950002/ Ref. 5
Bromelia serra Griseb.	FRP 98-17751-0/Horres 029 (FR)	H 029	EU780799/ Ref. 1	K2	EU219692/ Ref. 2	DQ084699/ Ref. 4	DQ084622/ Ref. 4	AY950019/ Ref. 5
Canistrum fosterianum L.B.Sm.	FRP 86-16991-3/Zizka 927 (FRP)	H 047	EU780840/ Ref. 1	K6	EU219697/ Ref. 2	AF188773/ Ref. 3	DQ084618/ Ref. 4	AY950024/ Ref. 5
Chevaliera cariocae (L.B.Sm.) L.B.Sm. & W.J. Kress	KEW 2001-1722/Chase 23820 (K)	KEW 23820	EU780825/ Ref. 1		EU780881/ Ref. 1	EU780857/ Ref. 1	EU780869/ Ref. 1	EU780845/ Ref. 1
Cryptanthus glaziovii Mez	HEID 102583/Schulte 010601-3 (FR)	H 215	EU780806/ Ref. 1		EU219683/ Ref. 2	DQ084701/ Ref. 4	DQ084635/ Ref. 4	AY950010/ Ref. 5
Deinacanthon urbanianum (Mez) Mez	FRP 98-17786-0/Horres 018 (FRP)	H 018	EU780800/ Ref. 1	K1	EU219690/ Ref. 2	AF188781/ Ref. 3	DQ084607/ Ref. 4	AY950017/ Ref. 5
Edmundoa lindenii (Regel) Leme	HEID 105009/Schulte 010601-4 (FR)	H 213	EU780831/ Ref. 1		EU219685/ Ref. 2	DQ084704/ Ref. 4	DQ084631/ Ref. 4	AY950012/ Ref. 5
Fascicularia bicolor (Ruiz & Pav.) Mez	FRP 98-16846-3/Zizka 1790 (FR)	H 006a	EU780802/ Ref. 1	K3	EU219696/ Ref. 2	AF188775/ Ref. 3	DQ084605/ Ref. 4	AY950023/ Ref. 5
Fernseea itatiaiae (Wawra) Baker	HEID 102174/Horres 067 (FR)	H 067b	EU780801/ Ref. 1	K9	EU219672/ Ref. 2	DQ084705/ Ref. 4	DQ084633/ Ref. 4	AY949999/ Ref. 5
Greigia spec. nov.	FRP 99-19040/Grant 19040 (FR)	H 157	EU780804/ Ref. 1		EU219687/ Ref. 2	DQ084710/ Ref. 4	DQ084601/ Ref. 4	AY950014/ Ref.1
Greigia mulfordii L.B.Sm.	-/Till 13090 (W)	H 111	EU780805/ Ref. 1		EU219689/ Ref. 2	DQ084709/ Ref. 3	DQ084600/ Ref. 3	AY950016/ Ref. 5
Hohenbergia eriostachia Mez	KEW 1972-1470/Chase 23818 (K)	KEW 23818	EU780841/ Ref. 1		EU780882/ Ref. 1	EU780858/ Ref. 1	EU780870/ Ref. 1	EU780846/ Ref. 1
Neoglaziovia variegata (Arruda) Mez	FRP 97-16794-3/ Zizka 1105 (FRP)	H 052	EU780813/ Ref. 1	K7	EU219724/ Ref. 2	AF188763/ Ref. 3	DQ084614/ Ref. 4	AY950051/ Ref. 5
Ochagavia elegans R.Phil.	FRP 98-16852-3/Horres 23a (FR)	H 23a	EU780803/ Ref. 1		EU219679/ Ref. 2	AF 188778/ Ref. 3	DQ084603/ Ref. 4	AY950006/ Ref. 5
Orthophytum disjunctum L.B.Sm.	KEW 1975-3132/Chase 23816 (K)	KEW 23816	EU780808/ Ref. 1	K1	EU780883/ Ref. 1	EU780859/ Ref. 1	EU780871/ Ref. 1	EU780847/ Ref. 1

(*continued on next page*)

Table 1 (*continued*)

Species	Accession no. living collection/ herbarium specimen	DNA-isolate no.	GenBank No./reference-No.					
			PRK	Clone (PRK)	*atp*B-*rbc*L spacer	*trn*L intron	*trn*L-*trn*F spacer	*mat*K, 3′*trn*K
Orthophytum maracasense L.B.Sm.	KEW 1979-3587/Chase 23817 (K)	KEW 23817	EU780809/ Ref. 1	K1	EU780884/ Ref. 1	EU780860/ Ref. 1	EU780872/ Ref. 1	EU780848/ Ref. 1
Orthophytum supthutii E.Gross & Barthlott	HEID 102160/Barthlott & Supthut 10315 (HEID)	H 223	EU780807/ Ref. 1	K10	EU219695/ Ref. 2	DQ084713/ Ref. 4	DQ084572/ Ref. 4	AY950022/ Ref. 5
Portea grandiflora Philcox	KEW 1977-760/Chase 23822 (K)	KEW 23822	EU780839/ Ref. 1		EU780885/ Ref. 1	EU780861/ Ref. 1	EU780873/ Ref. 1	EU780849/ Ref. 1
Portea leptantha Harms	FRP 99-18222-3/Schulte 060901-1 (FR); Zizka 1055 (FRP)	H 239	EU780838/ Ref. 1	K1	EU219725/ Ref. 2	DQ084714/ Ref. 4	DQ084621/ Ref. 4	AY950052/ Ref. 5
Quesnelia arvensis Mez	KEW 1975-3122/Chase 23821 (K)	KEW 23821	EU780835/ Ref. 1	K1	EU780886/ Ref. 1	EU780862/ Ref. 1	EU780874/ Ref. 1	EU780850/ Ref. 1
Quesnelia edmundoi L.B.Sm.	FRP 92-10483-3/Zizka 964 (FRP)	H 050	EU780837/ Ref. 1	K2	EU219719/ Ref. 2	AF188769/ Ref. 4	DQ084616/ Ref. 4	AY950046/ Ref. 5
Quesnelia lateralis Wawra	FRP 90-10484-0/Zizka 1554 (FRP)	H 051	EU780836/ Ref. 1	K3	EU219720/ Ref. 2	AF188771/ Ref. 3	DQ084615/ Ref. 4	AY950047/ Ref. 5
Streptocalyx poeppigii Beer	FRP 94-13845-4/Horres & Schulte 201101-5 (FR)	H 267	EU780830/ Ref. 1	K1	EU219677/ Ref. 2	DQ084719/ Ref. 4	DQ084598/ Ref. 4	AY950004/ Ref. 5
Ursulaea tuitensis (Magana & E.J.Lott) Read & Baensch	FRP s.n./Horres 033 (FR) voucher DNA	H 033	EU780828/ Ref. 1	K3	EU219700/ Ref. 2	DQ084720/ Ref. 4	DQ084625/ Ref. 4	AY950027/ Ref. 5
Wittrockia superba Lindm.	FRP 93-12641-0/Horres & Schulte 050401-8 (FR)	H 049	EU780832/ Ref. 1	K7	EU219698/ Ref. 2	AF188767/ Ref. 3	DQ084611/ Ref. 4	AY950025/ Ref. 5
Puyoideae								
Puya chilensis Molina	KEW 1988-8221/Chase 23824 (K)	KEW 23824	EU780798/ Ref. 1		EU780887/ Ref. 1	EU780863/ Ref. 1	EU780875/ Ref. 1	EU780851/ Ref. 1
Puya densiflora Harms	HEID 103568/Horres 076 (FR)	H 076	EU780797/ Ref. 1	K2	EU219670/ Ref. 2	DQ084716/ Ref. 4	DQ084564/ Ref. 4	AY949997/ Ref. 5
Puya ferruginea (Ruiz & Pav.) L.B.Sm.	KEW 1992-880/Chase 23826 (K)	KEW 23826	EU780796/ Ref. 1	K1	EU780889/ Ref. 1	EU780865/ Ref. 1	EU780877/ Ref. 1	EU780853/ Ref. 1
Puya mirabilis (Mez) L.B.Sm.	HEID 103731/Horres 060 (FR)	H 060	EU780794/ Ref. 1		EU219671/ Ref. 2	AF188793/ Ref. 3	DQ084562/ Ref. 4	AY949998/ Ref. 5
Puya raimondii Harms	KEW 1988-133/Chase 23825 (K)	KEW 23825	EU780795/ Ref. 1		EU780888/ Ref. 1	EU780864/ Ref. 1	EU780876/ Ref. 1	EU780852/ Ref. 1

the vouchered taxa analyzed (including GenBank Accession numbers) is presented in Table 1.

2.2. DNA extraction, amplification and sequencing

Total genomic DNA was extracted from fresh or lyophilized leaf material using a modified cetyl trimethylammonium bromide (CTAB) procedure (Doyle and Doyle, 1987) as described in Horres et al. (2000). To a large extent, raw genomic DNA from our former studies, stored at the Grunelius Möllgaard laboratory at the Senckenberg Research Institute, Frankfurt/Main, was used to generate the sequence data. For 10 taxa genomic DNA was obtained from the DNA Bank at the Royal Botanic Garden, Kew, UK (Table 1).

For amplification of PRK final reaction volumes of 25 µl were prepared from 22.5 µl Taq Master Mix containing 2.5 mM $MgCl_2$ (ReddyMix, ABgene), 0.25 µl of each primer [0.1 µg/µl], 0.5 µl BSA [0.04%], 1 µl DNA template [25 ng/µl], and 0.5 µl double-distilled water. Thermal cycling conditions were as follows: 1 cycle of 2 min at 94 °C, followed by 38 cycles of 1 min at 94 °C, 1 min at 50 °C, and 2 min at 72 °C, followed by one cycle of 7 min at 72 °C.

PRK primers (prk622f: 5′-CAG CAA TGA GGT TAA ATT TGC ATG GA-3′ and prk1069r: 5′-GAA AAT CTG C(AG)T GCT TCA GCA TTT G-3′) were designed to be fairly specific for one paralog of PRK (paralog 1) of Bromeliaceae (Barfuss et al., unpublished data). Primer prk622f is nested near the 3′ end of PRK exon 2 and prk1069r is positioned within exon 5 (Fig. 1). Products were cleaned from solution by using a PCR purification kit (NucleoSpin Extract, Macherey & Nagel) according to the manufacturer's protocol and eluted in 30 µl elution buffer. Sequencing reactions were performed according to the ABI Prism Big Dye 3.1 terminator cycle sequencing protocol (Applied Biosystems). Amplification primers were used as sequencing primers. In several cases internal primers (prk735f: 5′-CTG CAG ATC CGC AGA AGA AAT ATG C-3′; prk889r: 5′-GGG TAT GAG CAT GTC AAT TTC CTC CC-3′) positioned in exon 4 were used additionally. A study assessing the phylogenetic utility of PRK in Bromeliaceae including primer design, PCR optimization and analyses of different portions of the gene will be published elsewhere (Barfuss et al., unpublished data). The sequences were generated on an ABI 377 automated sequencer at the Royal Botanic Gardens, Kew. PCR and sequencing of the plastid regions were according to the protocols described earlier (*trn*L: Horres et al., 2000; *trn*L-*trn*F: Horres et al., 2007; *mat*K, 3′*trn*K: Schulte et al., 2005; *atp*B-*rbc*L: Schulte and Zizka, 2008).

In 70.8% of the accessions PRK raw electropherogram data obtained from direct sequencing included overlapping signals, which indicated the presence of heterozygotes, duplicated loci, or contamination with unspecific other PCR products. In these cases,

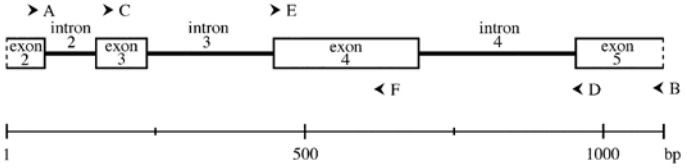

Fig. 1. Diagram of phosphoribulokinase (PRK) gene, paralog 1, exon 3 to exon 5. The location of primers is indicated by arrows. (A) prk622f; (B) prk1069r; (C) prk672f; (D) prk961r; (E) prk735f; (F) prk889r.

cloning was carried out. Amplified fragments of PRK were cloned using pGEM-T Easy Vector System (Promega) following the manufacturer's protocol. Successfully transformed colonies were sampled for PCR by inoculating PCRs with transformed cells as template. PCR products were cleaned and sequenced as described above.

For *Acanthostachys strobilacea* the target sequence could not be obtained employing the primers prk622f and prk1069r. For this taxon more specific primers were designed by the first author against the sequences already obtained from the other bromeliad taxa. Primer prk672f (5′-CAC AGC CTT GAA AGC ATC AAA′-3′) is nested near the 5′ end of exon 3, primer prk961r (5′-CAT CTC CAG CAT CGA AAC CT-3′) is located near the 5′ end of exon 5 (Fig. 1). Sequences generated using these primers were approx. 250 bp shorter than those obtained with prk622f and prk1069r.

2.3. Paralogy and heterozygosity

In spite of the use of fairly specific primers, an additional paralog of the target copy of PRK was identified in six species of Bromelioideae (*Aechmea warasii*, *Ananas comosus*, *Cryptanthus bahianus*, *Cryptanthus glaziovii*, *Hohenbergia stellata* and *Quesnelia liboniana*). Although being similar in length to the target copy of PRK, the paralog was easily distinguished in the sequence alignment from the target copy, since the two copies were hardly alignable due to considerable differences within both the intron and the exon parts. Nevertheless, BLAST searches (Altschul et al., 1997) of the paralog found it most similar to the PRK gene in *Triticum aestivum* and other Poaceae, followed by dicot PRK sequences (e.g. *Geranium dissectum*, *Acer saccharum*). In taxa where only this paralog of the target copy was obtained initially, a further PCR effort was performed in which additional clones were sequenced. In doing so, the target copy could be recovered in only one case (*Cryptanthus glaziovii*). The remaining taxa were successfully replaced by other representatives of the respective genera.

To assess the degree of variability of the target copy of PRK (paralog 1) within clones of the same species, 5 clones each of 9 species (*Aechmea distichantha*, *Aechmea drakeana*, *Aechmea farinosa*, *Aechmea mertensii*, *Canistrum fosterianum*, *Deinacanthon urbanianum*, *Fernseea itatiaiae*, *Orthophytum supthutii*, and *Wittrockia superba*) were sequenced and analyzed. Comparisons between the clones revealed that although different versions of the target copy were retrieved in the majority of species, they differed in only a small number of point mutations (caused by the presence of heterozygots or *Taq* errors during PCR) and for several taxa in a few indels (caused by length difference of the two alleles of one locus; i.e. *Aechmea mertensii*, *Canistrum fosterianum*, *Fernseea itatiaiae*). Indels were confined to intron 3 and intron 4, ranging from 1 to 18 nt, with indels of 1 to 3 nt length prevailing. The different copies of the target copy were all resolved as monophyletic groups in preliminary analyses and thus most likely represented alleles or artificial PCR products (e.g. *Taq* errors). Therefore, for the purpose of this study, one of the cloned sequences of the target copy was selected at random for inclusion in the analyses.

2.4. Alignment of sequences, data congruence and phylogenetic analysis

Contiguous alignments were assembled and edited using DNAStar Seqman II, version 5.07 (Lasergene, Madison, Wisconsin, USA). Sequences were aligned with ClustalX version 1.81 (Thompson et al., 1997) followed by manual adjustments in MacClade 4.06 for OS X (Maddison and Maddison, 2003). Regions of uncertain homology were excluded from analysis. Indels were coded in a binary matrix applying the simple indel coding method (Simmons and Ochoterena 2000) using SeqState version 1.36 (Müller, 2005, 2006). The same program was used to calculate sequence statistics (e.g. sequence divergence, number of parsimony informative characters).

For phylogenetic reconstruction all genomic regions were analyzed individually (PRK intron 2; PRK intron 3; PRK intron 4; PRK exon 3; PRK exon 4; PRK exon 5; *mat*K; 3′*trn*K; *trn*L; *trn*L-*trn*F; *atp*B-*rbc*L) and in different combinations (PRK introns; PRK exons; PRK gene; all 5 plastid regions: 5cp; nuclear and plastid regions combined: PRK5cp), also considering the respective indel characters.

Congruence between the individual data partitions as well as between the different combinations was tested using the incongruence length difference (ILD) test (Farris et al., 1995) implemented as the partition homogeneity test in PAUP Version 4.0b10 (Swofford, 2002) employing 100 replicates (heuristic search, 10 random addition replicates, TBR branch swapping), saving a maximum of 1000 most parsimonious trees per replicate.

Maximum parsimony ratchet analyses (Nixon, 1999) were conducted in PAUP with command files generated with PRAP (Müller, 2003). For each of the twenty random addition replicates, 200 ratchet iterations were performed. Each iteration comprised 10 rounds of TBR swapping, saving one shortest tree, and the most parsimonious trees were used to compute the consensus trees. Statistic support of the clades was estimated by nonparametric bootstraping as implemented in PAUP. Bootstrap proportions (BP) were based on 1000 pseudoreplicates, each with 10 random taxon addition replicates followed by tree-bisection-reconnection (TBR) branch swapping saving no more than 1000 trees per pseudo replicate. Tree length, consistency indices (CI), and retention indices (RI) were calculated for trees from the separate and combined analyses.

2.5. Bayesian analysis

Bayesian inference of phylogeny was accomplished using Metropolis-coupled Markov chain Monte Carlo (MCMCMC) estimation of posterior probability distributions as implemented in MrBayes version 3.1.2 (Ronquist and Huelsenbeck, 2003). For the combined analysis the alignment (excluding ambiguously aligned regions) was divided into eight partitions (PRK exons; PRK introns; *mat*K; 3′*trn*K; *trn*L; *trn*L-*trn*F; *atp*B-*rbc*L; indelmatrix). To avoid overparameterization of the combined model the individual PRK exon and PRK intron regions were analyzed as a combined partition each. For each of the seven DNA partitions the best-fit model of evolution was inferred using ModelTest version 3.6 (Posada and Crandall 1998) based on the Akaike Information Criterion AIC (Table 2). For Bayesian analysis the best-fit model according to ModelTest that could be implemented in MrBayes was assigned to each of the seven DNA partitions (Table 2). These as well as the combined datasets (PRK, 5cp, PRK5cp) were analyzed considering the respective indel matrices. In the partitioned models the overall evolutionary rate was allowed to be different across partitions. Furthermore, the nucleotide models were allowed to be unique

Table 2
Best-fit models of DNA substitutions based on the Akaike Information Criterion for the DNA partitions analyzed with Baysian inference. MT, best-fit model selected by Modeltest. BI, best-fit model selected by Modeltest that could be implemented in MrBayes.

Data partition	MT	BI
PRK exons	K80+I+G	K80+I+G
PRK introns	HKY+G	HKY+G
*mat*K gene	TIM+I+G	GTR+I+G
3′*trn*K intron	K81 uf	HKY
*trn*L intron	K81 uf+I+G	GTR+I+G
*trn*L-*trn*F spacer	GTR+G	GTR+G
*atp*B-*rbc*L spacer	GTR+I+G	GTR+I+G

for each partition, i.e. the substitution model parameters were enabled to be independent across partitions, only branch length and topology remained linked between partitions.

For each analysis three independent MCMCMC runs were conducted starting all chains from different, randomly chosen trees. Four Metropolis-coupled chains with incremental heating were used. After initial preruns the heating parameter was set to 0.1 to improve mixing behavior of the chains. All MCMC analyses were performed for 1,000,000 generations, sampling values every 100th generation. As default, the first 2500 sampled generations from each run were discarded from the analysis as burnin (i.e. the number of generations before apparent stationarity). After each analysis run statistics were considered to explore whether convergence had been reached after the designated burnin phase. In all runs the plots of model likelihood against generation had leveled off after the preset burnin phase and were fluctuating around a stable value. In all cases, the potential scale reduction factors (Gelman and Rubin, 1992) were very close to one (to the second decimal), indicating that the runs had adequately converged. The standard deviation of split frequencies between the three independent runs had dropped far below 0.1, indicating that the tree samples from the different runs were sufficiently similar.

Calculations of the 50% majority rule consensus trees and the posterior probabilities (PP) of each branch were based upon all tree samples of the three independent runs gained after the designated burnin phase.

2.6. Evaluation of character transformation patterns

Character transitions of sepal symmetry (symmetric, asymmetric) and habit (central tank present or absent) were examined to explore the evolution of these characters in the subfamily. The information was mainly compiled from literature (for sepal symmetry: Smith and Downs, 1974, 1979; Smith 1988; Leme 1997; for habit: Smith and Downs, 1974, 1979; Benzing 2000; supplemented by own observations). The selected characters were traced by overlying them onto the strict consensus tree of the parsimony analysis based on the overall datamatrix comprising nuclear as well as plastid data, including the coded indels.

3. Results

3.1. Variability of nuclear and plastid regions

The length of the analyzed PRK sequences from paralog 1 ranged between 713 (*Deinacanthon urbanianum*) and 1106 nt (*Aechmea mertensii*) due to considerable length mutations within the intron parts. Intron 3 and intron 4 were especially variable in sequence length, ranging from 113 to 416 nt and 148 to 416 nt, respectively. Within the introns three regions were identified, in which an accurate primary homology assessment was not possible and which therefore were excluded from analysis (intron 3: one region, intron 4: two regions), resulting in an overall alignment of the PRK sequence data of 888 nt length. Sequence statistics are summarized in Table 3.

Within the five plastid regions studied, the two spacer regions (*atp*B-*rbc*L, *trn*L-*trn*F) showed the highest variability in sequence length ranging from 614 to 793 and 188 to 346, respectively. Within the *atp*B-*rbc*L spacer a length variable polyT/polyA stretch precluded an unambiguous alignment and was therefore excluded from the analysis, leaving 846 characters in the alignment of the region. Likewise, a hypervariable region within the *trn*L-*trn*F region was excluded from the analysis, resulting in an alignment of 314 characters for the region. The combined alignment from all five plastid regions comprised 3393 characters in the final dataset (i.e. excluding regions of ambiguous alignment).

The combination of the nuclear region PRK and the five plastid regions led to an overall alignment of 4281 characters, excluding ambiguous regions. In total, the overall alignment contained 0.3% missing data. The proportion of variable and potentially informative characters in the analyzed data matrices was highest within the PRK introns with 44.7% and 20.6%, respectively, followed by the PRK exons with 26.4% and 12.4%, respectively. Among the five plastid regions, the proportion of variable characters ranged from 7.1% to 11.7%, being highest within the introns (*trn*K, *trn*L).The proportion of potentially informative characters within the plastid dataset was lowest within *mat*K and *atp*B-*rbc*L with 2.6% and highest within the intron regions (*trn*K, *trn*L) with 5.4% and 4.7%, respectively. Nevertheless, *mat*K

Table 3
Comparison of sequence statistics between all nuclear and chloroplast regions analyzed individually (Part A) and in different combinations (Part B) for the 48 taxon set of Bromelioideae. Char., number of characters in the alignment matrix (excluding hotspots); length range, actual sequence length in nucleotides (minimal and maximal observed value, including hotspots); mean length, mean of all observed sequence lengths (standard deviation given in brackets); % divergence, pairwise sequence distance in percent (uncorrected *P* distance), overall mean, lowest and highest scores in brackets); % var., percentage of variable characters; % inform., percentage of parsimony informative indels. *Minimal value due to missing data for *Acanthostachys strobilacea* (A.c.). The sequence was obtained using primer pairs nested within exon 3 and exon 5. Minimal values without A.c.: 33, 86, 64 for intron 2, exon 3 and exon 5, respectively.

Data partition	Char.	Length range	Mean length	% Divergence	% Var.	% Inf.	No. indels	% Inf. indels
(A) Individual								
	a	b	b	b	a	a	a	a
PRK intron 2	74	0*–56	50.4 (8.0)	4.7 (0.0–17.6)	37.8	14.9	33	33.3
PRK intron 3	142	113–416	213.4 (36.5)	10.1 (0.6–25.8)	48.6	22.5	7	28
PRK intron 4	270	148–310	264.0 (42.9)	8.2 (0.0–20.3)	44.4	21.1	39	35.9
PRK exon 3	86	9*–86	84.4 (11.0)	2.8 (0.0–22.2)	20.9	14	0	0
PRK exon 4	244	241–244	243.0 (0.4)	3.6 (0.0–7.8)	29.5	13.5	2	50
PRK exon 5	72	0*–72	69.8 (10.4)	2.2 (0.0–8.7)	22.2	6.9	4	50
*mat*K	1554	1524–1545	1533.7 (3.9)	0.7 (0.0–1.7)	7.5	2.6	5	20
*trn*K intron	185	169–185	179.9 (1.7)	1.4 (0.0–3.3)	11.4	5.4	2	0
*trn*L intron	494	467–484	474.4 (3.5)	1.0 (0.0–2.6)	11.7	4.7	10	30
*trn*L-*trn*F spacer	314	188–346	286.3 (19.0)	0.9 (0.0–3.7)	9.6	3.5	15	20
*atp*B-*rbc*L spacer	846	614–793	757.8 (34.2)	0.9 (0.0–2.0)	7.1	2.6	19	47.4
(B) Combined								
PRK introns	486	312–705	527.8 (60.0)	8.6 (0.5–18.1)	44.7	20.6	79	34.2
PRK exons	402	252–402	397.2 (21.3)	3.2 (0.0–6.2)	26.4	12.4	6	50
PRK	888	713–1106	925.0 (66.6)	6.0 (0.3–11.6)	36.4	16.9	85	35.3
5cp	3393	3091–3296	3232.1 (40.0)	0.8 (0.0–1.6)	8.4	3.1	51	31.4
PRK5cp	4281	3960–4345	4157.0 (75.1)	1.9 (0.2–3.5)	14.2	6	136	33.8

[a] Data refer to the matrix used in tree inference and exclude regions of ambiguous alignment.
[b] Data refer to complete matrix, including mutational hotspots.

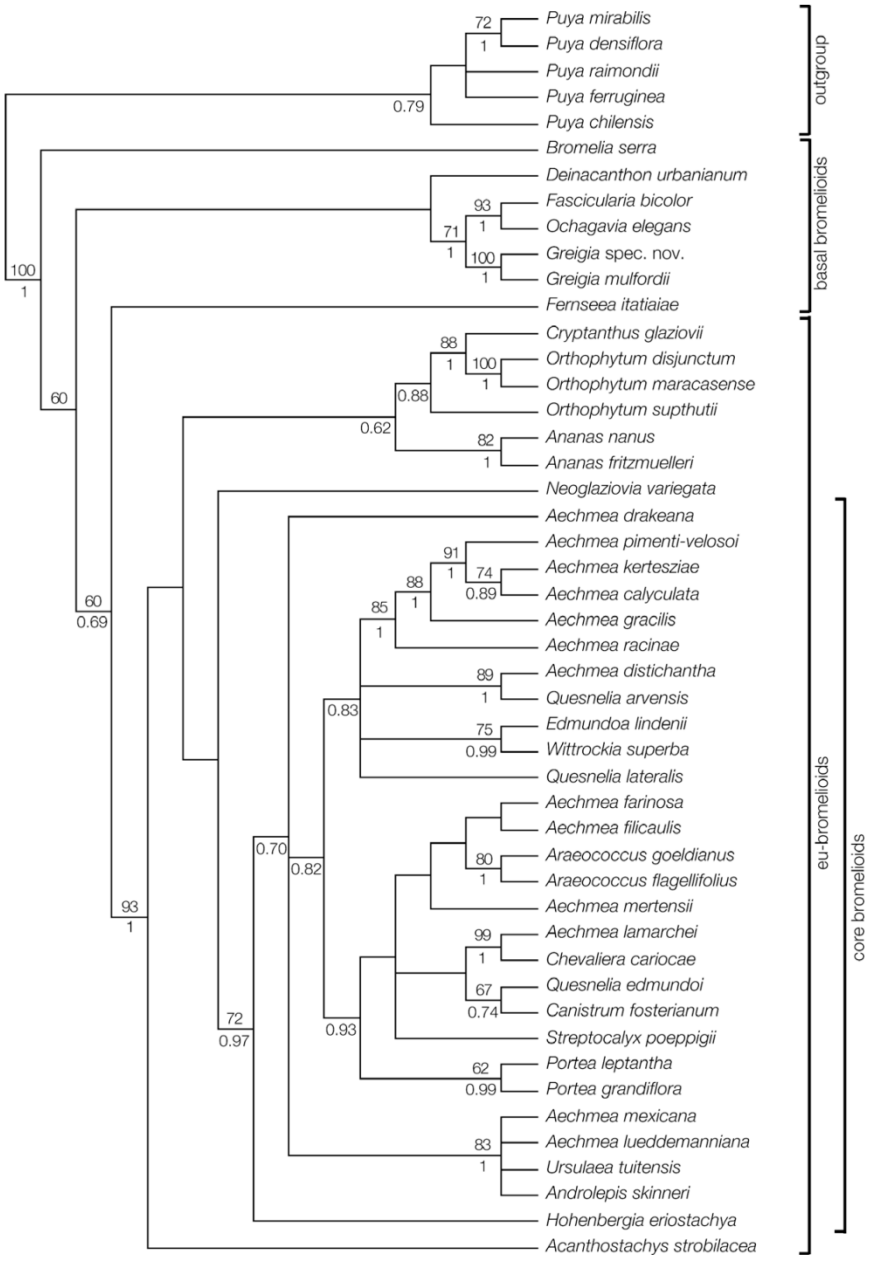

Fig. 2. Strict consensus tree of a parsimony ratchet analysis of the PRK region (intron 2 to exon 5) including coded indels. The analysis yielded 109 most parsimonious trees of 705 steps length (consistency index CI 0.67, retention index RI 0.70). Bootstrap support is given above branches, posterior probabilities from Bayesian analysis are indicated below branches.

contributed most to the total amount of potentially informative characters within the plastid dataset (40 of 106) due to its substantial length (1554 nt). The proportion of potentially informative characters was considerably higher within the combined PRK dataset than within the combined plastid dataset (16.9% vs. 3.1%). Of the 3393 characters of the combined dataset of nuclear and plastid regions, 14.2% were variable and 6.0% were parsimony informative.

Most indels were observed in the PRK introns, followed by the analyzed spacers and introns of the plastid genome and the lowest

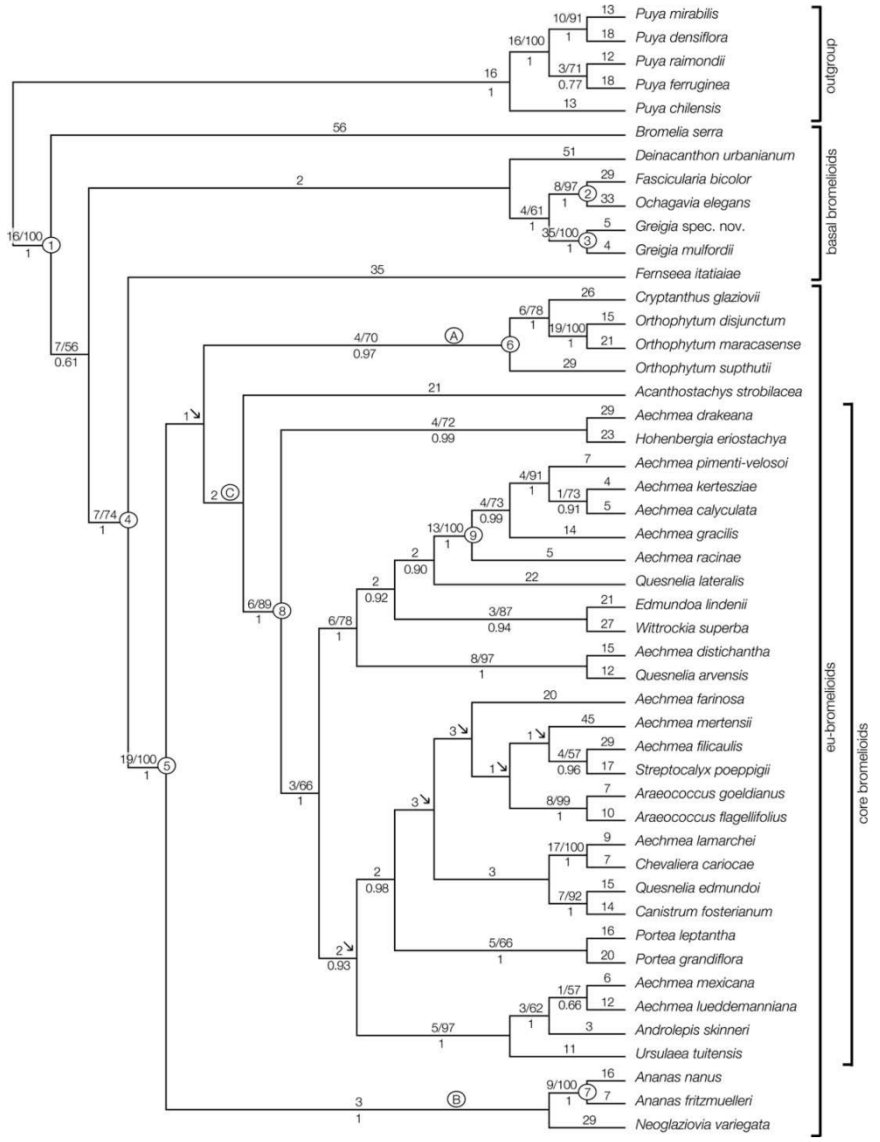

Fig. 3. One of 77 most parsimonious trees of a maximum parsimony analysis of PRK and five plastid regions (*mat*K gene, 3′*trn*K intron, *atp*B-*rbc*L spacer, *trn*L intron, *trn*L-*trn*F spacer), including coded indels, of 1168 steps length (consistency index CI 0.71, retention index RI 0.72). Branch length and bootstrap support are given above branches, posterior probabilities from Bayesian analysis are indicated below branches. Branches, that collapse in the strict consensus are marked with an arrow.

number of indels was found in the exon regions. Only 33.8% of the 136 indels of the overall matrix were potentially parsimony informative (Table 3).

3.2. Phylogenetic relationships

Partition homogeneity tests indicated that the different data partitions of the PRK region are not significantly incongruent (*P*-values in all pairwise comparisons >0.05) whereas within the plastid dataset two pairwise comparisons were significantly incongruent (*P*-values <0.01 for *atp*B-*rbc*L vs. *mat*K and *atp*B-*rbc*L vs. *trn*L). However, comparison of the topologies obtained from analysis of the different data partitions on a node-by-node basis, which may give a better assessment of congruence than the sometimes unreliable partition homogeneity test (Wiens, 1998; Dolphin et al., 2000; Reeves et al., 2001) did not result in any highly supported, incongruent relationships (BV > 70). Due to the general high level of congruence between the different data partitions, they were combined. Incongruence homogeneity tests for the different combined matrices (e.g. PRK exons vs. PRK introns, PRK vs. five plastid

regions) yielded no significant incongruence between the combined partitions (*P*-values > 0.08).

The trees obtained from the different datasets differed mainly in the degree of resolution, being lowest in the individual data partitions and highest in the combined dataset of the PRK region (Fig. 2) and the combined analysis of PRK and the five plastid regions (Fig. 3 and Tables 4, 5). The increased number of well-supported clades resulting from the analysis of the combined datasets relative to the separate data partitions gives further evidence to our assertion that the different datasets are broadly congruent. Therefore, the trees (MP, BI) obtained from the combined analysis of the total dataset of one nuclear and five plastid regions are considered as the best approximation of genetic relationships, and the MP strict consensus tree is described in the following (Fig. 3).

The 20 parsimony ratchet replicates of 200 iterations conducted with the overall dataset (PRK5cp) yielded a total of 77 most parsimonious trees with a length of 1,168 steps, a consistency index excluding uninformative characters of 0.71, and a rescaled consistency index of 0.51 (Table 4).

The phylogenetic analyses corrobate the monophyly of subfamily Bromelioideae (BV 100, PP 1). Within the sister group of Bromelioideae *Puya* (Pitcairnioideae *s.l.*), *Puya chilensis* (subgen. *Puya*) is found in sister group position to a strongly supported clade comprising representatives of both subgenera (*Puya* and *Puyopsis*) (BV 100, PP 1), not supporting the current subgeneric concept of *Puya*.

The monophyly of Bromelioideae is highly supported (BV 100, PP 1, Fig. 3: node 1). *Bromelia* is resolved in sister group position to all other Bromelioideae, neverthess this relationship receives only weak nodal support (BV 56/PP 0.61). On the next branch, *Deinacanthon*, *Fascicularia*, *Ochagavia* and *Greigia* form a clade sister to a moderately supported branch comprising the remaining Bromelioideae (BV 74, PP 1). The position of *Deinacanthon* remains unclear as the depicted sister group relationship to *Fascicularia*/*Ochagavia* and *Greigia* receives no statistical support. *Fascicularia* together with *Ochagavia* (BV 97, PP 1, Fig. 3: node 2) as well as *Greigia* (BV 100, PP 1, Fig. 3: node 3) form highly supported clades, but the sister group position of the two clades is only weakly supported in the bootstrap analysis (BV 61, PP 1).

Fernseea is resolved as sister to the remaining Bromelioideae (informally referred to as eu-bromelioids in the following) with high statistical support (BV 100, PP 1, Fig. 3: node 4). The eu-bromelioids split into a trichotomy. Branch A depicts *Orthophytum* as paraphyletic, with *Cryptanthus* being nested within the moderately supported clade (BV 70, PP 0, 97, Fig. 3: node 6). *Orthophytum supthutii* is found in sister group position to the remainder of the clade (BV 78, PP 1).

Branch B shows a highly supported *Ananas* clade (BV 100, PP 1, 3: node 7) together with *Neoglaziovia*, without gaining statistical support. Branch C depicts *Acanthostachys* in sister group position to a large and well-supported clade representing the core bromelioids (BV 89, PP 1, Fig. 3: node 8). The core bromelioids are well resolved, nevertheless relationships between supported clades remain largely uncertain due to a lack of nodal support. *Aechmea* and *Quesnelia* are shown as highly polyphyletic, their representatives being found in different positions within the core bromelioids. Among the different moderately to highly supported clades the following ones are noteworthy: (1) A moderately supported *Ortgiesia* clade (BV 73, PP 1, Fig. 3, node 9), consisting of *Aechmea* subgen. *Ortgiesia* with *Aechmea racinae* (subgen. *Lamprococcus*) in sister group position. (2) A highly supported "Nidularioid"-clade comprising *Edmundoa* and *Wittrockia* (BV 87, PP 0, 94). (3) A highly supported *Podaechmea* clade (BV 97) uniting *Ursulaea* and *Androlepis* with two species of *Aechmea* subgen. *Podaechmea* (BV 97, PP 1).

Table 4
Results of maximum parsimony analysis of different data partitions. Trees, number of most parsimonious trees; TL, length of shortest trees; CI, consistency index; RC, rescaled consistency index; No. of nodes, number of nodes present in the strict consensus tree.

Data partition	Trees	TL	CI	RC	No. of nodes
(A) Individual					
PRK intron 2	2379	85	0.82	0.60	2
PRK intron 3	3438	123	0.76	0.24	8
PRK intron 4	3798	271	0.71	0.53	7
PRK exon 3	1184	25	0.72	0.58	4
PRK exon 4	3524	121	0.65	0.50	7
PRK exon 5	1159	26	0.81	0.70	0
matK	8	144	0.88	0.79	18
trnK intron	21	26	0.89	0.83	6
trnL intron	966	92	0.80	0.62	10
trnL-trnF spacer	1912	53	0.89	0.88	3
atpB-rbcL spacer	1379	101	0.8	0.68	14
(B) Combined					
PRK introns	1692	505	0.70	0.50	23
PRK exons	366	186	0.63	0.47	17
PRK	705	109	0.67	0.48	37
5cp DNA	827	449	0.78	0.62	27
PRK5cp	77	1168	0.71	0.51	39

Table 5
Statistical support of selected nodes in the phylogenetic analysis of different datasets. Combined, PRK and 5 cp regions; nuclear, PRK; plastid, 5 cp regions (*matK* gene, 3′*trnK* intron, *atpB-rbcL* spacer, *trnL* intron, *trnL-trnF* spacer).

Node[a]	Statistical support (BV/PP)		
	Combined	Nuclear	Plastid
1. Monophyly of Bromelioideae	100/1	100/1	99/1
2. Monophyly of *Ochagavia/Fascicularia* clade	97/1	93/1	<50/ n.p.
3. Monophyly of *Greigia*	100/1	100/1	100/1
4. *Fernseea* sister to eu-bromelioids	74/1	60/0.69	n.p.
5. Monophyly of eu-bromelioids	100/1	93/1	98/1
6. Monophyly of *Orthophytum/Cryptanthus* clade	70/0.97	<50/ 0.88	n.p.
7. Monophyly of *Ananas*	100/1	82/1	100/1
8. Monophyly of core bromelioids	89/1	72/0.97	n.p.
9. Monophyly of *Ortgiesia* clade (including *Ae. racinae*)	100/1	85/1	99/1

[a] Node numbers refer to nodes in Fig. 3. n.p., node not present in the respective tree.

4. Discussion

4.1. Phylogenetic utility of PRK

Previous molecular studies in Bromeliaceae relied on plastid DNA data only, which exhibited an extraordinary low sequence divergence within subfamily Bromelioideae (Horres et al., 2000, 2007; Schulte et al., 2005). The low-copy nuclear gene PRK exhibits a significantly higher level of variation than that of any plastid region used in phylogenetic studies of Bromeliaceae so far. For example, the proportion of potentially parsimony informative characters obtained for the five plastid regions used in this study ranged from 2.6% to 5.4% whereas PRK yielded 16.9%. The estimates of phylogeny of Bromelioideae based on PRK data alone (888 characters) exhibited a higher resolution and support than those based on single plastid regions as well as the combination of up to five plastid regions (comprising 3393 characters). This study demonstrates that the examined PRK regions are of high utility in resolving phylogenetic relationships within Bromelioideae and are well capable of resolving relationships at intergeneric level. The intron regions are also expected to be of great value in resolving intrageneric relationships due to their high variability.

As has been demonstrated within this study special care has to be taken in discriminating between paralogous and orthologous regions. In Bromelioideae two highly divergent paralogs of PRK have been retrieved that were easily distinguishable in the assessment of homology. Furthermore cloning revealed highly similar copies of the target copy of PRK (paralog 1) within single accessions which could stem from either heterozygosity, PCR artifacts or recent paralogy. Preliminary analysis demonstrated that interspecific variation exceeded intraspecific variation in all cases, i.e. the copies of PRK formed monophyletic groups concordant with the species they were retrieved from. Thus, even if the different copies represented recent paralogy, the analyses indicate that possible gene duplication events postdated speciation. Therefore, the analysis of a random choice of these copies is regarded as appropriate approach to infer the phylogeny of Bromelioideae. The phylogenetic reconstructions obtained from the nuclear region PRK are fully congruent with results obtained from the five plastid regions, not indicating any conflicts, and possess a higher resolution, thus making the target region a valuable phylogenetic tool.

4.2. Phylogenetic relationships

One severe obstacle in the understanding of the evolution of Bromelioideae has been the difficulty in assigning the phylogenetic position of their genera, especially the assignment of putatively basal and advanced groups within the subfamily (e.g. Smith, 1934; Pittendrigh, 1948; Smith and Downs, 1979; Benzing, 2000). Molecular studies so far all had to contend with an exceedingly low sequence variability of plastid markers leading to poorly resolved phylogenies and often suffered from poor taxonomic sampling. Based on molecular studies with a thorough sampling relying on more than two plastid markers several putatively basal genera within the subfamily could be distinguished as well as a core group comprising the more advanced bromelioids (Schulte et al., 2005; Schulte and Zizka, 2008).

The inclusion of nuclear sequence data in the phylogenetic reconstruction of Bromelioideae significantly improves resolution as well as support for the revealed intergeneric relationships. The phylogenetic reconstructions including the nuclear region PRK now give reasonable evidence that the six genera *Bromelia*, *Deinacanthon*, *Greigia*, *Ochagavia/Fascicularia* and *Fernseea* represent early divergent lineages within the subfamily, and thus confirm the results of our earlier studies based on different plastid loci (Schulte et al., 2005; Schulte and Zizka, 2008).

The sister group relationship of *Bromelia*, a genus comprising terrestrial xerophytes, to the remainder of the subfamily is resolved for the first time in a molecular study with a thorough sampling, especially of putatively basal bromelioids, albeit receiving only weak statistical support. Although the genus has been regarded as an early divergent line within the subfamily before (e.g. Pittendrigh, 1948; Givnish et al., 2004, 2007; Schulte et al., 2005; Schulte and Zizka, 2008), up to now its position among the basal bromelioids has remained speculative, either because other putatively basal lineages of the subfamily where not represented in the molecular studies or because of a lack of resolution (Givnish et al., 2004, 2007; Horres et al., 2000, 2007; Schulte et al., 2005; Schulte and Zizka, 2008). Nevertheless, the relationships between early divergent bromelioids as indicated by the inclusion of the nuclear marker PRK in the molecular dataset still need to be corroborated by additional molecular evidence.

According to the molecular phylogeny the monotypic genus *Deinacanthon* represents a distinct lineage within the basal bromelioids and its separation from *Bromelia* (Mez, 1896; Smith, 1988) appears justified.

The molecular tree shows an affinity between the xeric genera *Ochagavia/Fascicularia* and the mesophytic *Greigia*. The three genera share several traits with *Puya*, the sister group of Bromelioideae, which are discussed as ancestral in the subfamily (e.g. C3 photosynthesis, distribution types; terrestrial life form; Schulte et al., 2005).

The sister group position of *Fernseea* to the remainder of the subfamily, as already indicated by plastid data alone (Schulte et al., 2005), is further corroborated by the inclusion of nuclear data and now is highly supported. *Fernseea*, a small genus of terrestrial plants of high mountain habitats in southeastern Brazil, has been discussed earlier as possible remnant of the migration of Bromelioideae between the Andes and eastern Brazil, the latter being today's center of diversity within the subfamily (Schulte et al., 2005).

Within the highly supported clade comprising the remaining Bromelioideae, several lineages can be discerned from the more advanced core bromelioids.

The *Orthophytum/Cryptanthus* clade gives the first evidence for the paraphyly of *Orthophytum*, with *Cryptanthus* being nested within the clade. Both genera are endemic to southeastern Brazil and comprise terrestrial or lithophytic xerophytes. Recent morphological studies within the genus *Orthophytum* indicate that *Orthophytum supthutii* belongs to a more primitive group that merits its own generic status (Louzada, 2008). Within our molecular phylogeny including nuclear data, *Orthophytum supthutii* is found in sister group position to a clade comprising *Cryptanthus* and the remaining *Orthophytum* species, thus supporting the view of Louzada (2008).

The morphologically well characterized genus *Ananas* forms another highly supported clade, whereas the sister group position of *Neoglaziovia* to the *Ananas* clade receives no nodal support. The phylogenetic position of *Acanthostachys*, which is depicted as sister group of the core bromelioids in the consensus trees of the analysis of the overall data matrix, remains unclear.

Within the core bromelioids resolution has improved compared to the molecular studies based on plastid data alone but relationships between the different moderately to highly supported clades still remain uncertain. Once again, the polyphyly of the large and highly problematic genus *Aechmea* becomes evident, with members of the genus being found in different positions of the clade, grouping together with members of several genera such as *Quesnelia*, *Streptocalyx*, *Chevaliera*, *Androlepsis* and *Ursulaea*. This supports findings based on AFLPs (Horres et al., 2007) and plastid data alone (Schulte et al., 2005; Schulte and Zizka, 2008) as well as morphological studies (Faria et al., 2004). Two highly supported groups, the *Ortgiesia* clade and the *Podaechmea* clade have been consistently found in the AFLP and the plastid study as well and merit further investigation (Schulte et al., 2005). According to Givnish et al. (2004, 2007), the clade representing the core bromelioids is rather young with an estimated age of the crown group of around 5 Ma. This clade obviously underwent a rapid diversification, especially within the Atlantic rain forest of southeastern Brazil, leading to the current taxonomic difficulties. Due to the low sequence divergence displayed by the group, relationships are still not revealed satisfactorily. The integration of further informative nuclear regions into the phylogenetic reconstruction promises to shed more light on phylogeny and evolution of this highly diverse clade.

4.3. Evolution of sepal symmetry

Sepals in Bromelioideae are often asymmetric, with one sepal lobe larger than the other, the enlarged lobe often gaining a wing-like appearance. Most genera comprise species that possess either distinctly asymmetric or rather symmetric sepals. Smith (1988) used sepal symmetry in the identification of bromelioid genera without attaching further taxonomic value to the character. The inferred evolution of sepal symmetry indicates that symmetric se-

pals are ancestral within the subfamily. As the reconstruction of character evolution based on the molecular MP strict consensus tree shows, the sepals of the basal lineages of Bromelioideae can be regarded as primarily symmetric (Fig. 4). Asymmetric sepals are found within the *Ananas* clade and within the core bromelioids. To elucidate whether the occurence of asymmetric sepals within these two clades stem from a common ancestor or were gained independently, the position of the *Ananas* clade needs to be further resolved by the inclusion of additional molecular data. Nevertheless, tracing of character transitions onto the molecular tree indicates that asymmetric sepals are ancestral within the core bromelioids and that several reversals to symmetric sepals occurred within this lineage. Interestingly several clades are found in which more than one genus possesses symmetric sepals indicating that these reversals stem from common ancestry. This is the case for the genera *Edmundoa*, *Wittrockia*, *Nidularium* which belong to the Nidularioid clade (Schulte et al., 2005; Schulte and Zizka, 2008, this study), *Ursulaea* and *Hohenbergiopsis* belonging to the Central American *Podaechmea* clade (Schulte et al., 2005; Schulte and Zizka, 2008, this study), and *Canistrum fosterianum* and *Quesnelia edmundoi* which form another clade (Fig. 4). Within the genus *Quesnelia* symmetric sepals are the exception and might indicate wrong taxonomic concepts as the molecular data suggests. The evolutionary explanation for the occurrence of symmetric and asymmetric sepals within *Araeococcus* requires further investigation with a broader taxon sampling.

4.4. Evolution of the tank habit

The eco-morphological types of Tietze (1906), Pittendrigh (1948) and Benzing (2000) reflect the progression of a suite of characters that are partly evolving interdependently (e.g. reduction of root system and increase of water uptake capacity via leaf trichomes). Within Bromelioideae the more primitive eco-morphological types can be discerned from the most advanced type by the absence/presence of a central tank, which allows the plant to collect large quantities of water and organic material in an external repository. Whereas representatives of the more primitive eco-morphological types are almost exclusively terrestrials or lithophytes, representatives of the most advanced type (type III in all classifications) exhibit the highest flexibility in life form (epiphytic, lithophytic, less often terrestrial).

The reconstruction of the evolution of a central tank within the subfamily shows an unexpectedly clear pattern. The absence of a central tank is inferred as ancestral within the subfamily. The molecular phylogeny implies a single origin of the central tank within the subfamily namely ancestral to core bromelioids. Within the core bromelioids all genera are characterized by the presence of the central tank, which thus can be regarded as a synapomorphy for the clade. Singular exceptions are found within the clade in genera that are predominantly tank-forming. The character reconstruction implies that the tank habit was lost again in some taxa, as for example in *Araeococcus flagellifolius*, where the rosette forms an apically closed pseudobulb. The tank habit thus possesses a much lower evolutionary lability within the Bromelioideae than previously assumed, on the contrary, it appears to be highly conserved. This demonstrates the considerable advantage of the evolution of the central tank within the group. By gaining this key innovation, bromelioids promptly gained a high ecological flexibility. The rapid diversification of the core bromelioids, which is indicated by the molecular data, may have been triggered by the evolution of the central tank, enabling the group to conquer the most diverse hab-

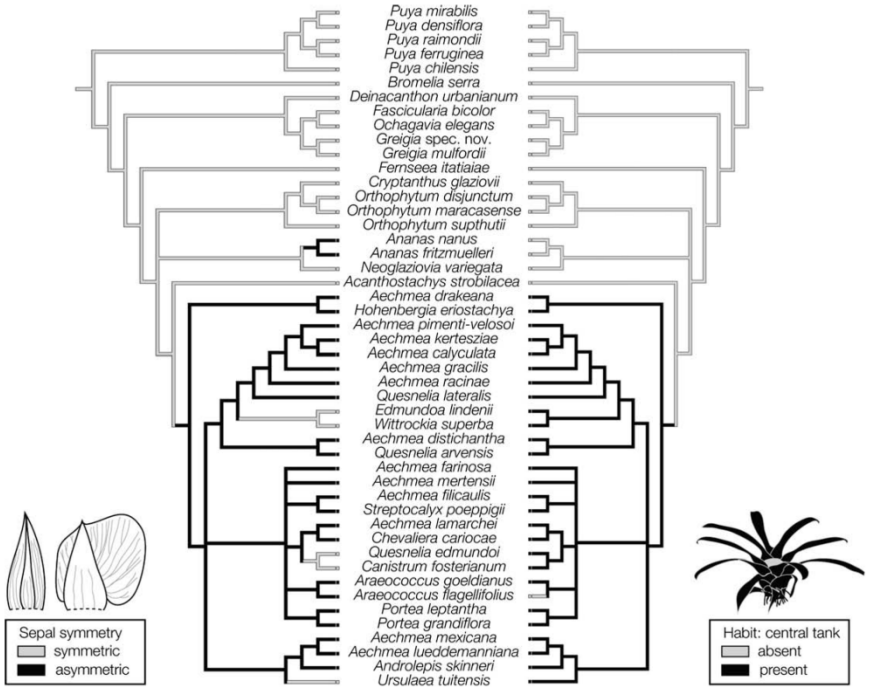

Fig. 4. Most parsimonious reconstruction of the evolution of sepal symmetry (on the left) and tank habit (on the right) in Bromelioideae, based on the strict consensus tree from a parsimony ratchet analysis of PRK and five plastid regions (*mat*K gene, 3′*trn*K intron, *atp*B-*rbc*L spacer, *trn*L intron, *trn*L-*trn*F spacer), including indels.

itats, being highly independent of water and nutrition supply from the substrate.

Acknowledgments

The authors are grateful to Mark Chase, Jim Clarkson, Ben Davis, and Edith Kapinos, Royal Botanic Gardens, Kew, Rosabelle Samuel, Hermann Voglmayr, and Walter Till, Department of Systematic and Evolutionary Botany, University of Vienna, Vienna for methodical support (PRK) and valuable discussion. The authors thank Kai Müller, Nees Institute, University of Bonn, for support in data analysis. We acknowledge financial support provided by the Deutsche Forschungsgemeinschaft (ZI 557/2-1, 2-2, 5-1 and 7-1, SCHU 2426/1-1), Marga & Kurt Möllgaard-Stiftung, Hermann Willkomm-Stiftung and "Freunde und Förderer der J.W. Goethe-Universität". The authors also acknowledge funding from the Hessian initiative for the development of scientific and economic excellence (LOEWE) at the Biodiversity and Climate Research Center BIK-F, Frankfurt/Main. Plant material was kindly supplied by the Palmengarten Frankfurt/Main, the Botanical Gardens of the Universities Heidelberg, Berlin-Dahlem, Kassel and from the Royal Botanic Gardens, Kew. The valuable comments of two anonymous referees are appreciated.

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Chapter 3

Optimizing eight nuclear DNA markers for phylogenetic studies in recently diverged angiosperms: A case study in Bromeliaceae subfamily Tillandsioideae

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Keywords: glucose-6-phosphate isomerase, cytosolic; malate synthase; low-copy nuclear DNA; nitrate reductase 1, [NADH]; ITS nuclear ribosomal DNA; phosphoribulokinase; phytochrome C; RNA polymerase II, beta subunit; xanthine dehydrogenase

Running Title: Nuclear DNA markers in Tillandsioideae (Bromeliaceae)

Status: in preparation, intended to be submitted to *American Journal of Botany*

Contribution: data collection, data analyses, manuscript writing

Abstract

Comparative studies of nuclear DNA sequences within Tillandsioideae show that several nuclear markers are able to provide more information and a higher degree of resolution in phylogenetic trees than previously used plastid markers. However, their utility depends not only on sequence variability, but also on methodological challenges, especially when traditional Sanger-sequencing is used. The presence of strong secondary structures in ITS nrDNA creates problems in amplification and sequencing. Therefore it is not recommended as a suitable marker for phylogenetic investigations of Bromeliaceae, also because of low resolution at deeper nodes. However, ITS nrDNA regions might be useful for delimiting species. Amplified fragments of the genes malate synthase (*MS*) and RNA polymerase II, beta subunit (*RPB2*) are not helpful due to their relatively short length and restricted number of PICs. Glucose-6-phosphate isomerase (*PGIC*) needs to be further investigated, but sequence length and variation found in the two species sequenced suggests it will be suitable at generic and higher taxonomic levels. Likewise, nitrate reductase 1, [NADH] (*NIA*) and xanthine dehydrogenase (*XDH*) need further investigations; the first might be suitable for lower taxonomical levels, while the second would be better suited for higher levels. Phosphoribulokinase (*PRK*) seems to be one of the most promising markers, but length differences between bromeliad taxa and indels within alleles of heterozygous accessions made it the most difficult to edit and align and thus time-consuming. For phytochrome C (*PHYC*) generating sequences and the editing/alignment have been straight forward. *PRK* and *PHYC* are useful nuclear markers and able to provide considerable resolution in phylogenetic trees, though some relationships remain uncertain.

Introduction

Despite rapid technical progress in molecular systematics, the goal of reconstructing fully resolved and well-supported phylogenetic trees in many species-rich and/or recently diverged angiosperm groups remains challenging (e.g., Lukas, 2010; Safer, 2011). Many molecular studies still analyze only plastid DNA markers or the multi-copy internal transcribed spacer nuclear ribosomal DNA (ITS nrDNA) of the 18S-5.8S-26S nrDNA repeat unit (e.g., Gruenstaudl & al., 2009; Russell & al., 2010). Availability of universal primers for recently characterized plastid markers (Shaw & al., 2005; 2007) and the ITS nrDNA region (White & al., 1990; Sun & al., 1994; Blattner, 1999; Gruenstaeudl & al., 2009) as well as the most advantageous single-copy nature of plastid DNA and the usually well-homogenized ITS nrDNA (concerted evolution) made them more suitable for phylogenetic studies. Usually these markers are simple to amplify, directly sequence, and align across lower taxonomic levels. Also, design of new primers became much easier due to the steadily increasing number of available sequences and complete plastid genomes publically available. However, a major challenge of commonly used plastid DNA and nrDNA markers is resolution of phylogenetic relationships at all taxonomic levels, even when many markers are combined. A common strategy to solve these problems is the use of low-copy nuclear DNA (e.g., Strand & al., 1997; Sang, 2002; Chapman 2007; Álvarez & al., 2008), but these markers are often avoided for several reasons. Inheritance of plastid DNA is predominantly uniparental and maternal in plants, whereas nuclear DNA is biparentally inherited, although due to conversion nrDNA can become uniparental. Processes like incomplete lineage sorting, hybridization, coalescence times longer than species ages, and introgression may complicate interpretation of results (Piñeiro & al., 2009; Willyard & al., 2009). More difficulties arise when polyploid taxa or multi-copy nuclear genes and gene families are investigated (e.g., Sang & al., 2004). Analyzing and interpreting such data can be problematic because multiple alleles and paralogs are present. However, for particular evolutionary questions and certain plant groups with low variation in plastid DNA or nrDNA sequences, more rapidly evolving low-copy nuclear DNA markers are a promising solution. An increasing number of studies published over the recent years have utilized such sequences to investigate phylogenetic relationships (e.g., Emshwiller & Doyle, 1999; Cronn & al., 2002; Yockteng & Nadot, 2004; Vaezi & Brouillet, 2009), reticulate evolution (e.g., Ma & al., 2010; Russell & al., 2010) and hybrid speciation (e.g., Sang & Zhang, 1999; Tank & Sang, 2001; Clarkson & al., 2010), or to uncover genome evolution (e.g., Wendel, 2000).

The pineapple family (Bromeliaceae), with more than 3,100 species assigned to 58 genera, represents a large and ecologically important group of monocots for which infrafamilial classification and phylogenetic relationships have been problematic for many years. Several earlier studies have tried to create well-resolved trees using one or a few plastid markers with limited success (Terry & al., 1997a, b; Horres & al., 2000; Crayn & al., 2004). Accompanying studies focusing on the largest subfamilies Tillandsioideae (Barfuss & al., 2005) and Bromelioideae (Schulte & Zizka, 2008) as well as Bromeliaceae in general (Givnish & al., 2011), both including a wider selection of taxa and plastid markers, were able to resolve the main phylogenetic units within the family but still showed low support for some deeper nodes and comparatively little sequence divergence between genera and species. Efforts to sequence and combine many plastid spacer regions that are supposed to evolve more rapidly (Shaw & al., 2005, 2007) than previously sequenced markers for Bromeliaceae (Barfuss & al., 2005; Schulte & Zizka, 2008;

Givnish & al., 2011: *atpB-rbcL*, *trnK-matK*, *ndhF*, *psbA-trnH*, *rpL32-trnL*, *rps16* intron, and 5'*trnL*-3'*trnL-trnF*) are of limited success in gathering sufficient phylogenetic information at lower taxonomic levels (M. H. J. Barfuss, unpublished data: *atpI-atpH*, *psbD-trnT*, *psbJ-petA*, *rpoB-trnC*, *trnD-trnT*, 3'*trnV-ndhC*,and *trnQ*-5'*rps16*). Low sequence divergence associated with plastid DNA sequences in phylogenetic studies of bromeliads were initially mainly attributed to generally low substitution rates in the bromeliad plastid genome (e.g., Gaut & al., 1992; Givnish & al., 2004, 2007). Insights from the recently published eight-locus plastid analysis of Bromeliaceae have shown that the family itself is old (100 My), but extant lineages of bromeliads arose less than 20 My ago (Givnish & al., 2011). The most species-rich clades are less than 5 My old. These results support the hypothesis that extant lineages have undergone recent and rapid radiations and subsequent diversification in newly formed ecological niches and not that substitution rates are lower than in other angiosperm lineages. The first molecular studies of bromeliads that include low-copy nuclear DNA sequences were recently published by Schulte & al. (2009) for Bromelioideae (phosphoribulokinase, *PRK*), Chew & al. (2010) for *Tillandsia* subgenus *Tillandsia* (internal transcribed spacer 2, ITS 2, external transcribed spacer, ETS nrDNA), Sass & Specht (2010) for *Aechmea* (RNA polymerase II, beta subunit, *RPB2*, glyceraldehyde-3-phosphate dehydrogenase, *G3PDH*, ETS nrDNA), Jabaily & Sytsma (2010) for *Puya* (phytochrome C, *PHYC*), and Versieux & al. (2012) for *Alcantarea* (*FLORICAULA*/*LEAFY*, *FLO*/*LFY*). However, relationships within Bromeliaceae still remain inadequately understood because these markers were applied only to restricted sets of taxa.

In this study we aim (1) to investigate primers for several nuclear DNA markers of angiosperms with focus on monocots and Bromeliaceae especially; (2) to resolve lower taxonomic units of subfamily Tillandsioideae down to species groups; (3) to evaluate relationships of recently diverged species; and (4) to detect hybrids, possible hybrid speciation, and reticulate evolution. We also selected the ITS nrDNA since the complete region was never used in a phylogenetic study for a large set of samples above the subgeneric level.

Materials and methods

Selection of taxa

For initial primer trials, three sets of few individuals of Tillandsioideae (2, 8, or 13) were selected. Larger sets of samples (64, 72, or 114) with a minimum of two accessions per major clade in other analyses were chosen based on analyses of seven plastid markers (Barfuss & al., 2005) to demonstrate variability of lineages. The complete set of samples (444) was finally analyzed for the most promising nuclear DNA markers. A sample list including botanical authors is given in Supplementary Data of chapter 5; accession details can be obtained from the first author upon request.

Extraction of high-quality DNA

Initially the CTAB procedure established by Doyle and Doyle (1987) modified for minipreps (1.5 or 2 mL tubes) was used. Due to the high content of polysaccharides, polyphenols, and other secondary metabolites in more than ⅔ of the Tillandsioideae species studied, this method did not yield sufficient amounts of high quality DNA, which is necessary to amplify suitable quantities of low-copy nuclear DNA. Therefore total genomic DNA was extracted from fresh or silicagel dried leaf tissue using the altered CTAB procedure of Tel-Zur & al. (1999) with further

reagent modifications, and with adjustments within the protocol, mainly implemented to allow the samples to be processed as minipreps. Most important chemical changes from the original protocol include (1) the addition of 1% PVP-40 into the Sorbitol buffer, (2) the increase of the CTAB concentration to 3% and the NaCl concentration to 3 M in the high-salt CTAB buffer, and (3) the addition of PVP-40 into the extraction buffer. In most cases this method yielded superior quantities of high molecular weight DNA which could be used immediately for PCR. When good DNA was visualized on 1% TAE agarose gels, but initial PCR trials were not successful, DNA was further purified or newly extracted using a modified CTAB/DNeasy Plant Mini Kit (QIAGEN) procedure. Protocols have been developed either starting from DNA dissolved in TE buffer or after CTAB extraction before (and therefore not doing a) precipitation with isopropanol, to avoid precipitating of any unwanted contaminants (mainly polyphenolic compounds) which could inhibit Taq polymerase activity. This combined method yielded much better DNA extracts than using the QIAGEN kit alone. Detailed protocols of all modifications of the extraction procedures can be obtained from the first author upon request.

Selection of nuclear markers

The ITS nrDNA region (e.g., White & al., 1990; Sun & al., 1994; Gruenstaeudl & al., 2009; Chew & al., 2010) and seven low-copy nuclear DNA markers were selected and tested: malate synthase (*MS*; e.g., Lewis & Doyle, 2001, 2002), RNA polymerase II, beta subunit (*RPB2*; e.g., Denton & al., 1998; Oxelman & al., 2004; Thomas & al., 2006; Sass & Specht, 2010), glucose-6-phosphate isomerase, cytosolic (*G6PIC* = phosphoglucose isomerase C, *PGIC*; Terauchi & al., 1997; Ford & al., 2006), nitrate reductase 1, [NADH] (*NR1* = nitrate reductase apoenzyme, *NIA* : e.g., Howarth & Baum, 2002), xanthine dehydrogenase (*XDH*; Górniak & al., 2010; Morton, 2011), phosphoribulokinase (*PRK*; e.g., Lewis & Doyle, 2002; Thomas & al., 2006; Schulte & al., 2009), and phytochrome C (*PHYC*; e.g., Mathews & Donoghue, 1999; Samuel & al., 2005; Russell & al., 2010). Regions were selected based on the availability of primers and/or DNA sequences available in GenBank to be able to design primers for Bromeliaceae. The primary target was to obtain one or more genomic markers that are either homogenized (ITS nrDNA) or can be treated as effectively single-copy (other nuclear markers) to be sequenced directly. Other parameters for the selection of markers were (1) performance of primers and reagents for both amplification (e.g., primer annealing, primer-dimer formation) and sequencing (e.g., homogeneity), (2) occurrence of homopolymers and microsatellites, which can give problems in sequencing, (3) amplicon length, and (4) number of potential phylogenetically informative characters (PICs). The genetic structure of each low-copy nuclear DNA region was determined from the assembled RefSeq genome of *Oryza sativa* and is summarized in Table 1. Positions of primers selected finally are given in Figure 1, A–H. All sequences generated for this study will be deposited in GenBank (http://www.ncbi.nlm.nih.gov/genbank).

Nomenclature of primers

Positions of primers on chromosomes and within the coding sequence (CDS) of the genes were determined by BLAST searches, both within the entire database and the annotated genes of the assembled RefSeq genome of *Oryza sativa* (Tables 3–10). This was possible because all primers are located in exons at least for ¾ of their length. Names of previously published primers were taken from their primary publication, and those of newly generated or modified ones were derived from the CDS position of the last primer base at the 3' end. If a modified primer ended at the same position as the original one, a number after a hyphen was attached.

Letter extensions after a hyphen indicate primers specific to a particular plant group (an = angiosperms, mo = monocots, br = bromeliads).

Table 1. Attributes of the low-copy nuclear genes based on GenBank information from the assembled RefSeq genome of *Oryza sativa*. Amplified regions are highlighted in red. Annotation of malate synthase and RNA polymerase II, beta subunit was incomplete or wrong and therefore done manually aided with reference sequences of *Arabidopsis thaliana* (*RPB2*) or *Zea mays* (*MS*).

	malate synthase (*MS*)		**RNA polymerase II, beta subunit (*RPB2*)**		**glucose-6-phosphate isomerase, cytosolic (*G6PIC*, *PGIC*)**	
	Chromosome: 4 (NC_008397) Gene: *MS* (Os04g0486950) mRNA: NM_001187064 Total range: 24,724,583-24,726,722 total length: 2131 (complement) Processed length: 1704 Protein product length: 567 Copy number: 1 Incomplete annotation in GenBank!		Chromosome: 3 (NC_008396) Gene: *RPB2* (Os03g0646800) mRNA: NM_001057305 Total range: 25,794,125-25,801,820 Total length: 7,696 Processed length: 3,555 Protein product length: 1184 Copy number: 1 Incomplete annotation in GenBank!		Chromosome: 6 (NC_008399) Gene: *PGIC-b* (Os06g0256500) mRNA: NM_001063851 Total range: 8,151,838-8,158,107 Total length: 6,270 Processed length: 1,704 Protein product length: 567 Copy number: 2 Duplicate gene: *PGIC-a* (Os03g0776000)	
exon 1	**1-375**	**375**	**1-234**	**234**	**1-51**	**51**
intron 1	376-459	84	235-1792	1558	52-205	154
exon 2	**460-785**	**326**	**1793-2014**	**222**	**206-282**	**77**
intron 2	786-1048	263	2015-2085	71	283-375	75
exon 3	**1049-1379**	**331**	**2086-2376**	**291**	**358-439**	**82**
intron 3	1380-1468	89	2377-2582	206	440-512	73
exon 4	**1469-2140**	**672**	**2583-2780**	**198**	**513-560**	**48**
intron 4			2781-2907	127	561-862	302
exon 5			**2908-2972**	**65**	**863-1018**	**156**
intron 5			2973-3054	82	1019-1093	75
exon 6			**3055-3141**	**87**	**1094-1190**	**97**
intron 6			3142-3254	113	1191-1288	98
exon 7			**3255-3375**	**121**	**1289-1331**	**43**
intron 7			3376-3466	91	1332-1721	390
exon 8			**3467-3511**	**45**	**1722-1789**	**68**
intron 8			3512-3587	76	1790-1879	90
exon 9			**3588-3725**	**138**	**1880-1964**	**85**
intron 9			3726-3837	112	1965-2044	80
exon 10			**3838-3910**	**73**	**2045-2093**	**49**
intron 10			3911-3989	79	2094-2314	221
exon 11			**3990-4066**	**77**	**2315-2387**	**73**
intron 11			4067-4148	82	2388-2465	78
exon 12			**4149-4256**	**108**	**2466-2526**	**61**
intron 12			4257-4374	118	2527-3460	934
exon 13			**4375-4491**	**117**	**3461-3527**	**67**
intron 13			4492-4790	299	3528-3596	69
exon 14			**4791-4946**	**156**	**3597-3653**	**57**
intron 14			4947-5046	100	3654-3746	93
exon 15			**5047-5148**	**102**	**3747-3800**	**54**
intron 15			5149-5302	154	3801-3883	83
exon 16			**5303-5428**	**126**	**3884-4012**	**129**
intron 16			5429-5503	75	4013-4538	526
exon 17			**5504-5581**	**78**	**4539-4602**	**64**
intron 17			5582-5656	75	4603-4712	110
exon 18			**5657-5752**	**96**	**4713-4786**	**74**
intron 18			5753-5858	106	4787-4869	83
exon 19			**5859-5974**	**116**	**4870-4923**	**54**
intron 19			5975-6047	73	4924-5042	119
exon 20			**6048-6117**	**70**	**5043-5111**	**69**
intron 20			6118-6196	79	5112-5740	629
exon 21			**6197-6250**	**54**	**5741-5830**	**90**
intron 21			6251-6328	78	5831-6002	172
exon 22			**6329-6472**	**144**	**6003-6110**	**108**
intron 22			6473-6563	91	6111-6222	112
exon 23			**6564-6863**	**300**	**6223-6270**	**48**
intron 23			6864-7046	183		
exon 24			**7047-7469**	**423**		
intron 24			7470-7582	113		
exon 25			**7583-7696**	**114**		

	nitrate reductase 1, [NADH] (*NR1*, *NIA*)		**xanthine dehydrogenase (*XDH*)**		**phosphoribulokinase (*PRK*)**		**phytochrome C (*PHYC*)**	
	Chromosome: 8 (NC_008401) Gene: *NIA-a* (Os08g0468100) mRNA: NM_001068541 Total range: 23,121,355-23,126,236 Total length: 4,882 Processed length: 2,751 Protein product length: 916 Copy number: 3 Duplicate genes: *NIA-b* (Os02g0770800) *NIA-c* (Os08g0468700)		Chromosome: 3 (NC_008396) Gene: *XDH* (Os03g0429800) mRNA: NM_001056955 Total range: 18,621,710-18,634,104 Total length: 12,395 Processed length: 4,110 Protein product length: 1,369 Copy number: 1		Chromosome: 2 (NC_008395) Gene: *PRK-a* (Os02g0698000) mRNA: NM_001054360 Total range: 29,588,876-29,590,869 Total length: 1,994 Processed length: 1,212 Protein product length: 403 Copy number: 2 Duplicate genes: *PRK-b* (Os04g0595700) complement: Os02g0697900 (partial)		Chromosome: 3 (NC_008396) Gene: *PHYC* (Os03g0752100) mRNA: NM_001057831 Total range: 31,768,179-31,772,647 Total length: 4,469 Processed length: 3,414 Protein product length: 1,137 Copy number: 1 Similar genes: *PHYA* (Os03g0719800) *PHYB* (Os03g0309200)	
exon 1	**1-1039**	**1039**	**1-142**	**142**	**1-545**	**545**	**1-2065**	**2065**
intron 1	1040-1147	108	143-1269	1127	546-838	293	2066-2169	104
exon 2	**1148-1288**	**141**	**1270-1369**	**100**	**839-923**	**85**	**2170-2986**	**817**
intron 2	1289-3226	1938	1370-1846	477	924-1012	89	2987-3379	393
exon 3	**3227-3459**	**233**	**1847-1955**	**109**	**1013-1097**	**85**	**3380-3673**	**294**
intron 3	3460-3544	84	1956-2595	640	1098-1267	170	3674-4231	558
exon 4	**3545-4882**	**1338**	**2596-4098**	**1503**	**1268-1512**	**245**	**4232-4469**	**238**
intron 4			4099-4938	840	1513-1742	230		
exon 5			**4939-5181**	**243**	**1743-1994**	**252**		
intron 5			5182-5291	110				
exon 6			**5292-5606**	**315**				
intron 6			5607-6859	1253				
exon 7			**6860-7099**	**240**				
intron 7			8000-7435	336				
exon 8			**7436-7720**	**285**				
intron 8			7721-7795	75				
exon 9			**7796-8017**	**222**				
intron 9			8018-8565	548				
exon 10			**8566-8727**	**162**				
intron 10			8728-8871	144				
exon 11			**8872-9006**	**135**				
intron 11			9007-10912	1906				
exon 12			**10913-11149**	**237**				
intron 12			11150-11247	98				
exon 13			**11248-11421**	**174**				
intron 13			11422-12152	731				
exon 14			**12153-12395**	**243**				
intron 14								
exon 15								
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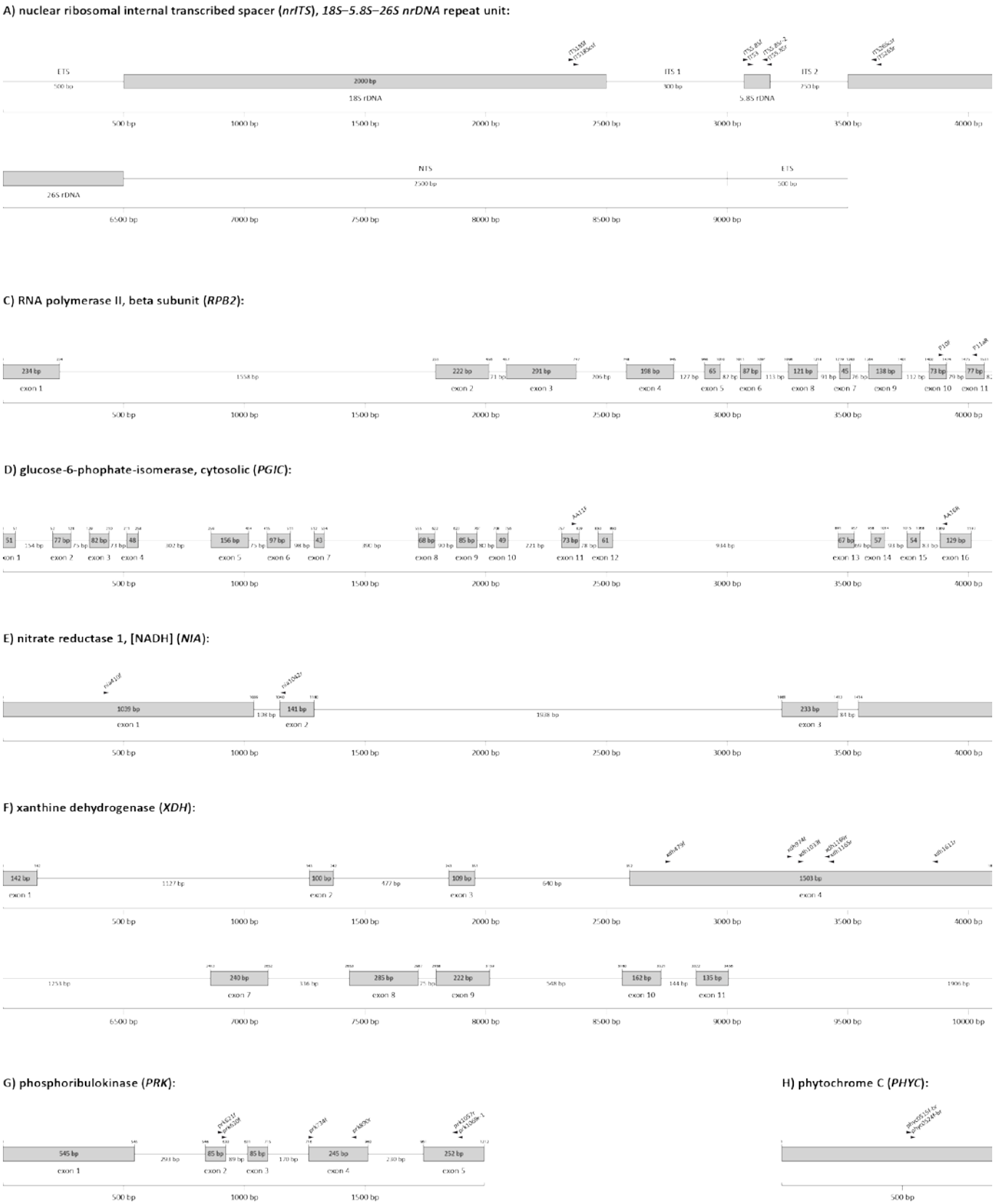
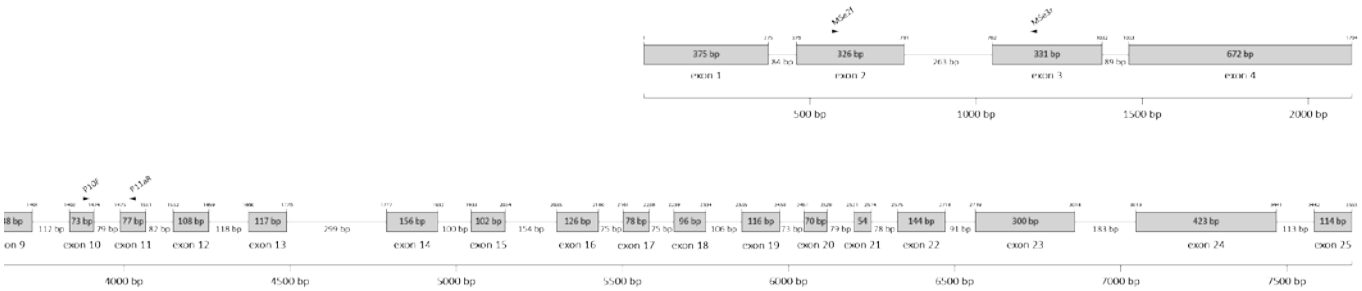
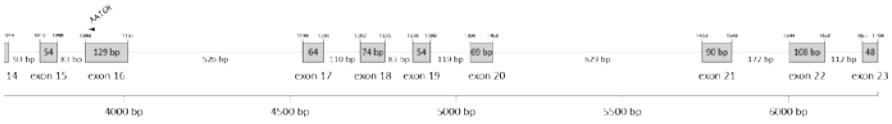
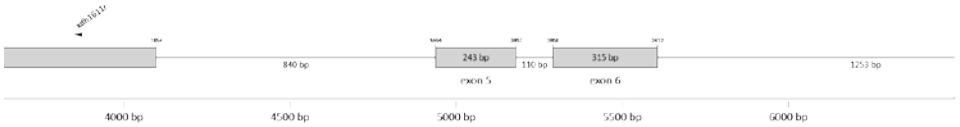


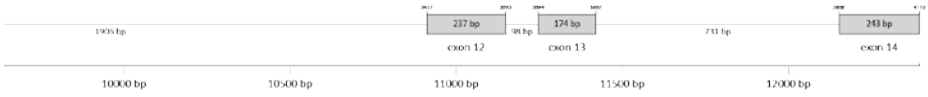
Figure 1. Genetic structure of each nuclear DNA region based on GenBank information from the assembled RefSeq genome of *Oryza sativa*. Approximate positions of primers finally used are indicated by arrow heads. Annotation of malate synthase and RNA polymerase II, beta subunit was incomplete or wrong and therefore done manually aided with reference sequences of *Arabidopsis thaliana* (*RPB2*) or *Zea mays* (*MS*).

B) malate synthase (*MS*):









(*PHYC*):

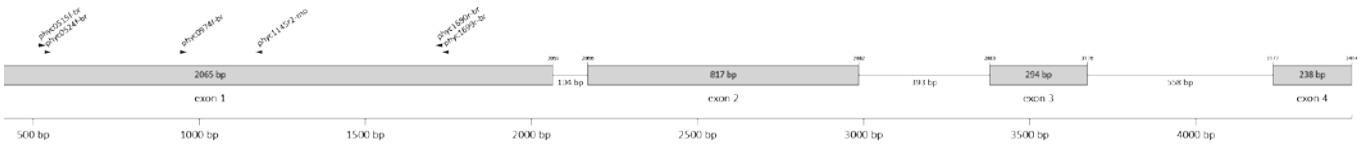


Table 2. Amplified markers, number of taxa studied, and PCR conditions of each marker.

marker	no. of taxa studied	PCR conditions
ITS nrDNA	114	1× 97°C for 2 min; 15× 99°C for 20 s, 64°C for 15 s, 72°C for 2 min; 20× 99°C for 15 s, 64°C for 10 s, 72°C for 2 min + 5 s/cycle; 1× 72°C for 7 min; 1× 4°C for ∞.
MS	13	1× 95°C for 2 min; 5× 95°C for 30 s, 60°C for 30 s, 72°C for 2 min; 30× 95°C for 30 s, 54°C for 30 s, 72°C for 2 min; 1× 72°C for 7 min; 1× 4°C for ∞.
RPB2	8	1× 95°C for 2 min; 40× 95°C for 30 s, 55°C for 30 s, 72°C for 1 min; 1× 72°C for 7 min; 1× 4°C for ∞.
PGIC	2	1× 95°C for 2 min; 35× 95°C for 30 s, 50°C for 30 s, 70°C for 2 min; 1× 70°C for 7 min; 1× 4°C for ∞.
NIA	71	1× 95°C for 2 min; 10× 95°C for 30 s, 65°C - 1°C/cycle for 30 s, 70°C for 2 min; 30× 95°C for 30 s, 58°C for 30 s, 70°C for 2 min; 1× 70°C for 7 min; 1× 4°C for ∞.
XDH	65	1× 95°C for 2 min; 10× 95°C for 30 s, 65°C - 1°C/cycle for 30 s, 70°C for 2 min; 30× 95°C for 30 s, 58°C for 30 s, 70°C for 2 min; 1× 70°C for 7 min; 1× 4°C for ∞.
PRK	444	1× 95°C for 2 min; 15× 95°C for 30 s, 62°C for 30 s, 70°C for 1 min 30 s; 20× 95°C for 30 s, 62°C for 30 s, 70°C for 1 min 30 s + 5 s/cycle; 1× 70°C for 7 min; 1× 4°C for ∞.
PHYC	444	1× 95°C for 2 min; 20× 95°C for 30 s, 59°C for 30 s, 70°C for 1 min 30 s; 20× 95°C for 30 s, 59°C for 30 s, 70°C for 1 min 30 s + 5 s/cycle; 1× 70°C for 7 min; 1× 4°C for ∞.

Table 3. Primers tested for the internal transcribed spacer of nuclear ribosomal DNA (ITS nrDNA). Highlighted primers performed best and were selected for PCR or sequencing. bp = base pairs.

name	sequence (5'–3')	position	length	direction	use/success	specificity	reference
primers initially aivailable							
17SE (AB101)	ACGAATTCATGGTCCGGTGAAGTGTTCG	18S nrDNA, 3' end	28 bp	forward	PCR unsuccessful	angiosperms	Sun & al., 1994
ITS-A	GGAAGGAGAAGTCGTAACAAGG	18S nrDNA, 3' end	22 bp	forward	primer design	angiosperms	Blattner, 1999
ITS5	GGAAGTAAAAGTCGTAACAAGG	18S nrDNA, 3' end	22 bp	forward	PCR unsuccessful	universal	White & al., 1990
ITS1	TCCGTAGGTGAACCTGCGG	18S nrDNA, 3' end	19 bp	forward	not used	universal	White & al., 1990
ITS-D	CTCTCGGCAACGGATATCTCG	5.8S nrDNA, 5' end	21 bp	forward	primer design	angiosperms	Blattner, 1999
ITS3	GCATCGATGAAGAACGCAGC	5.8S nrDNA	20 bp	forward	not used	universal	White & al., 1990
ITS2	GCTGCGTTCTTCATCGATGC	5.8S nrDNA	20 bp	reverse	PCR unsuccessful	universal	White & al., 1990
ITS-C	GCAATTCACACCAAGTATCGC	5.8S nrDNA	21 bp	reverse	PCR unsuccessful	angiosperms	Blattner, 1999
ITS-B	CTTTTCCTCCGCTTATTGATATG	26S nrDNA, 5' end	23 bp	reverse	not used	angiosperms	Blattner, 1999
ITS4	TCCTCCGCTTATTGATATGC	26S nrDNA, 5' end	20 bp	reverse	PCR unsuccessful	universal	White & al., 1990
26SE (AB102)	TAGAATTCCCCGGTTCGCTCGCCGTTAC	26S nrDNA, 5' end	28 bp	reverse	PCR unsuccessful	angiosperms	Sun & al., 1994
primers subsequently tested							
ITS18Sf	ACCGATTGAATGGTCCGGTGAAGTGTTCG	18S nrDNA, 3' end	29 bp	forward	PCR	angiosperms	Gruenstaeudl & al., 2009
ITS18Scsf	GAATGGTCCGGTGAAGTGTTCG	18S nrDNA, 3' end	22 bp	forward	sequencing	angiosperms	this study
ITS5c	AGAGGAAGGAGAAGTCGTAACAAGGT	18S nrDNA, 3' end	26 bp	forward	sequencing	angiosperms	this study; modified from “ITS5” & “ITS-A”
ITS5.8Sf	ACTCTCGGCAACGGATATCTCGGCTC	5.8S nrDNA, 5' end	26 bp	forward	PCR	angiosperms	Gruenstaeudl & al., 2009
ITS5.8Scsf	GACTCTCGGCAACGGATATCTCG	5.8S nrDNA, 5' end	23 bp	forward	sequencing	angiosperms	this study; modified from “ITS-D”
ITS-E	CGGCAACGGATATCTCGGCTC	5.8S nrDNA, 5' end	21 bp	forward	sequencing	angiosperms	Blattner 1999
ITS5.8Scsr	GATGCGTGACGCCCAGGCAG	5.8S nrDNA, 3' end	20 bp	reverse	sequencing	angiosperms	this study
ITS5.8Sr	ATGCGTGACGCCCAGGCAGACGTG	5.8S nrDNA, 3' end	24 bp	reverse	PCR	angiosperms	Gruenstaeudl & al., 2009
ITS5.8Sr-an	ATGCGTGACGCCCAGGCAGRCGTG	5.8S nrDNA, 3' end	24 bp	reverse	PCR	angiosperms	this study; modified from “ITS5.8Sr”
ITS5.8Scsr-2	TGACGCCCAGGCAGRCGTGC	5.8S nrDNA, 3' end	20 bp	reverse	sequencing	angiosperms	this study
26SE-2	CGGTTCGCTCGCCGTTACTA	26S nrDNA, 5' end	20 bp	reverse	sequencing	angiosperms	this study
ITS26Scsr	GGACGCTTCTCCAGACTACAATTCG	26S nrDNA, 5' end	25 bp	reverse	sequencing	angiosperms	this study
ITS26Sr	CTGAGGACGCTTCTCCAGACTACAATTCG	26S nrDNA, 5' end	29 bp	reverse	PCR	angiosperms	Gruenstaeudl & al., 2009

Table 4. Primers tested for malate synthase (*MS*). Only highlighted primers worked and were selected for PCR and sequencing. Position of primers was determined from the assembled RefSeq genome of *Oryza sativa*. bp = base pairs. CDS = coding sequence. Chr = chromosome.

name	sequence (5'–3')	CDS position (bp)	Chr position (bp)	length	direction	use/success	specificity	reference
primers initially tested								
ms400f	GGAAGATGRTCATCAAYGCNCTYAAYTC	exon 1, 329–356	24,726,367–24,726,394	28 bp	forward	unsuccessful	angiosperms	Lewis & Doyle 2001
ms356f	GGAAGATGRTMATCAAYGCRCTKAAYTC	exon 1, 329–356	24,726,367–24,726,394	28 bp	forward	unsuccessful	angiosperms	D. Springate, RBG Kew, unpublished; modified from "ms400f"
ms526f	GGACTATAAGCTTCCATGACCTC	exon 2, 455–477	24,726,162–24,726,184	23 bp	forward	unsuccessful	palms, some other monocots	Lewis & Doyle 2001
ms943r	GTCTTNACRTAGCTGAADATRTARTCCC	exon 3, 872–899	24,725,504–24,725,477	28 bp	reverse	unsuccessful	angiosperms	Lewis & Doyle 2001
ms1408r	CCARTTCTGVACBCKGCTGATCTCCGC	exon 4, 1,408–1,434	24,724,853–24,724,879	27 bp	reverse	unsuccessful	angiosperms	D. Springate, RBG Kew, unpublished
ms1488r	TTCRTAYYTNAKCCAYTGCCAGTTYTG	exon 4, 1,426–1,452	24,724,835–24,724,861	27 bp	reverse	unsuccessful	angiosperms	Lewis & Doyle 2001
primers subsequently tested								
ms356f-2	GGAAGATGRTCATCAAYGCNCTBAAYTC	exon 1, 329–356	24,726,367–24,726,394	28 bp	forward	unsuccessful	monocots	this study; modified from "ms400f"
ms428f (MSe2f)	GCTGGGARAACCTGATGARVGGCCA	exon 2, 404–428	24,726,211–24,726,235	25 bp	forward	PCR/sequencing	monocots	this study
ms553r (MSe2r)	CCGTCGAYGAGRATRTGGGCCTC	exon 2, 553–575	24,726,064–24,726,086	23 bp	reverse	unsuccessful	monocots	this study
ms837f (MSe3f)	CARATGRAYGAGATVCTNTACGAGCTG	exon 3, 811–837	24,725,539–24,725,565	27 bp	forward	unsuccessful	monocots	this study
ms960r (MSe3r)	GGARRTCGGAGTAGCTSCKCATGAAG	exon 3, 960–985	24,725,391–24,725,416	26 bp	reverse	PCR/sequencing	monocots	this study
ms1411r (MSe4r)	TGCCAGTTCTGVACBCKGCTGATCTC	exon 4, 1,411–1,436	24,724,851–24,724,876	26 bp	reverse	unsuccessful	monocots	this study; modified from "ms1488r"

Table 5. Primers tested for RNA polymerase II, beta subunit (*RPB2*). Only highlighted primers worked and were selected for PCR and sequencing. Position of primers was determined from the assembled RefSeq genome of *Oryza sativa*. bp = base pairs. CDS = coding sequence. Chr = chromosome.

name	sequence (5'–3')	CDS position (bp)	Chr position (bp)	length	direction	use/success	specificity	reference
P6F	TGGGGAATGATGTGTCCTGC	exon 11, 1,513–1,532	25,798,152–25,798,171	20 bp	forward	unsuccessful	angiosperms	Denton & al., 1998
P7F	CCYCGTAATACWTAYCARTCWGC	exon 17, 2,164–2,186	25,799,631–25,799,653	23 bp	forward	unsuccessful	angiosperms	Denton & al., 1998
P7R	CCCATGGCTTGCTTCCCCAT	exon 17 2,188–2,207	25,799,655–25,799,674	20 bp	reverse	unsuccessful	angiosperms	Denton & al., 1998
P10F	CARGARGATATGCCATGGAC	exon 23, 2,851–2,870	25,800,820–25,800,839	20 bp	forward	PCR/sequencing	angiosperms	Denton & al., 1998
P10R	CCCATRATACACTCAATGAGYTG	exon 23, 2,938–2,960	25,800,907–25,800,929	23 bp	reverse	unsuccessful	angiosperms	Denton & al., 1998
P11aR	GTGAATCTTGTCATCMACCATATGC	exon 24, 3,153–3,177	25,801,305–25,801,329	25 bp	reverse	PCR/sequencing	angiosperms	Denton & al., 1998

Table 6. Primers tested for glucose-6-phosphate isomerase, cytosolic (*PGIC*). Only highlighted primers worked and were used for PCR and sequencing. Position of primers was determined from the assembled RefSeq genome of *Oryza sativa*. bp = base pairs. CDS = coding sequence. Chr = chromosome.

name	sequence (5'–3')	CDS position (bp)	Chr position (bp)	length	direction	use/success	specificity	reference
AA11F (93.4)	TTYGCNTTYTGGGAYTGGGT	exon 11, 793–812	8,154,188–8,154,207	20 bp	forward	PCR/sequencing	universal	Ford & al., 2006 (Terauchi & al., 1997)
AA16F	ATGGARAGYAAYGGNAARGG	exon 16, 1,075–1,094	8,155,727–8,155,746	20 bp	forward	unsuccessful	angiosperms	Ford & al., 2006
AA16R	CCYTTNCCRTTRCTYTCCAT	exon 16, 1,075–1,094	8,155,727–8,155,746	20 bp	reverse	PCR/sequencing	angiosperms	Ford & al., 2006
AA21RM	CCCCAYTGRTCRAAIGARTTDATICCCCA	exon 21, 1,504–1,532	8,157,623–8,157,651	29 bp	reverse	unsuccessful	angiosperms	Ford & al., 2006
pgic1504r	CCCCAYTGRTCRAANGARTTDATNCCCCA	exon 21, 1,504–1,532	8,157,623–8,157,651	29 bp	reverse	unsuccessful	angiosperms	this study; modified from "AA21RM"
93.9H	TCIACICCCCAITGRTCTAAIGARTTIAT	exon 21, 1,510–1,538	8,157,629–8,157,657	29 bp	reverse	unsuccessful	universal	Terauchi & al., 1997
yamv	TCIACICCCCAITGRTCAAAIGARTTIAT	exon 21, 1,510–1,538	8,157,629–8,157,657	29 bp	reverse	unsuccessful	angiosperms	Ford & al., 2006; modified from "93.9H"
pgic1510r	TCNACNCCCCANTGRTCAAANGARTTNAT	exon 21, 1,510–1,538	8,157,629–8,157,657	29 bp	reverse	unsuccessful	angiosperms	this study; modified from "yamv"

Table 7. Primers tested for nitrate reductase 1, [NADH] (*NIA*). Highlighted primers performed best and were selected for PCR and/or sequencing. Position of primers was determined from the assembled RefSeq genome of *Oryza sativa*. bp = base pairs. CDS = coding sequence. Chr = chromosome.

name	sequence (5'–3')	CDS position (bp)	Chr position (bp)	length	direction	use/success	specificity	reference
primers initially tested								
NIA2F	TCBGTGATTACGACGCCGTGTCATGA	exon 2, 1,087–1,112	23,122,549–23,122,574	26 bp	forward	unsuccessful	angiosperms	Howarth & Baum 2002
NIA2R	GACCARAARCACCARCACCARTAYT	exon 3, 1,283–1,307	23,124,683–23,124,707	25 bp	reverse	unsuccessful	angiosperms	Howarth & Baum 2002
NIA3F	AARTAYTGGTGYTGGTGYTTYTGGTC	exon 3, 1,282–1,307	23,124,682–23,124,707	26 bp	forward	PCR/sequencing	angiosperms	Howarth & Baum 2002
NIA3R	GAACCARCARTTGTTCATCATDCC	exon 4, 1,414–1,437	23,124,899–23,124,922	24 bp	reverse	PCR/sequencing	angiosperms	Howarth & Baum 2002
primers subsequently tested								
nia410f (nia1f)	CTNATGCACCACGGNTTCATCAC	exon 1, 388–410	23,121,742–23,121,764	23 bp	forward	PCR/sequencing	angiosperms	this study
nia413f (nia1f-2)	CTNATGCACCACGGNTTYATCACCCC	exon 1, 388–413	23,121,742–23,121,767	26 bp	forward	PCR	angiosperms	this study
nia1042r (nia2r-3)	TGATKATRTAYTCYGGCTTRTACCACCA	exon 2, 1,042–1,069	23,122,504–23,122,531	28 bp	reverse	PCR/sequencing	angiosperms	this study
nia1073f (nia2f-2)	GGTACAAGCCRGARTAYATMATYAACGA	exon 2, 1,046–1,073	23,122,508–23,122,535	28 bp	forward	unsuccessful	angiosperms	this study
nia1108r (nia2r-2)	GTTGATGGGYARRATCTCSTCGTG	exon 2, 1,108–1,131	23,122,570–23,122,593	24 bp	reverse	unsuccessful	angiosperms	this study
nia1390r [1] (nia3r-2)	ACCATGACGTTCCAGATGAGCTTCTC	exon 3, 1,390–1,413 (*-1,415*)	23,124,790–23,124,815	26 bp	reverse	unsuccessful	angiosperms	this study

note: [1] italicized numbers indicate base positions that partly lie within introns (GT motive of intron start).

Table 8. Primers tested for xanthine dehydrogenase (*XDH*). Highlighted primers performed best and were selected for PCR and/or sequencing. Position of primers was determined from the assembled RefSeq genome of *Oryza sativa*. bp = base pairs. CDS = coding sequence. Chr = chromosome.

name	sequence (5'–3')	CDS position (bp)	Chr position (bp)	length	direction	use/success	specificity	reference
xdh422f (xdh4f-1)	CCYGGTTTYRTBATGTCVATGTATGC	exon 4, 397–422	18,624,350–18,624,375	26 bp	forward	PCR	monocots	this study
xdh437f (xdh4f-2)	GTCVATGTATGCVTTRYTRMGRTCAAG	exon 4, 411–437	18,624,364–18,624,390	27 bp	forward	PCR	monocots	this study
xdh479f (xdh4f-3)	TGARGARCARATYGAAGAWWGCCTTGC	exon 4, 453–479	18,624,406–18,624,432	27 bp	forward	PCR/sequencing	monocots	this study
xdh974f (xdh4intf-2)	GTTACCCATGTGGCGGAGCTTAATGC	exon 4, 949–974	18,624,902–18,624,927	26 bp	forward	sequencing	bromeliads	this study
xdh1033f-br (xdh4intf-1)	GTGCTTCTGTGAGACTGACACAGCTCC	exon 4, 1,007–1,033	18,624,960–18,624,986	27 bp	forward	sequencing	bromeliads	this study
xdh1165r-br (xdh4intr-1)	TAGGTCAGATATTGGACTAGCAGTACA	exon 4, 1,165–1,191	18,625,118–18,625,144	27 bp	reverse	sequencing	bromeliads	this study
xdh1169r-br (xdh4intr-2)	GGGTTTAGGTCAGATATTGGACTAGCAG	exon 4, 1,169–1,196	18,625,122–18,625,149	28 bp	reverse	sequencing	bromeliads	this study
xdh1611r (xdh4r-1)	CGRAAYTCHACCATYCCMCCWGGWGCA	exon 4, 1,611–1,637	18,625,564–18,625,590	27 bp	reverse	PCR/sequencing	monocots	this study
xdh1612r (xdh4r-2)	CGRAAYTCHACCATYCCMCCWGGWGC	exon 4, 1,612–1,637	18,625,565–18,625,590	26 bp	reverse	PCR	monocots	this study
xdh1880f (xdh5f-1)	GGTYACWGGDGARGCDGAATATRCTGA	exon 5, 1,854–1,880	18,626,647–18,626,673	27 bp	forward	unsuccessful	monocots	this study
xdh1883f (xdh5f-2)	ACWGGDGARGCDGAATATRCTGAYGA	exon 5, 1,858–1,883	18,626,651–18,626,676	26 bp	forward	unsuccessful	monocots	this study
xdh2386r[1] (xdh6r-1)	ACYTGDGTRGATGAWATCATRTGAAYTTC	exon 6, 2,386–2,412 (–*2,414*)	18,627,289–18,627,317	29 bp	reverse	unsuccessful	monocots	this study
xdh2390r[1] (xdh6r-2)	ACYTGDGTRGATGAWATCATRTGAA	exon 6, 2,390–2,412 (–*2,414*)	18,627,293–18,627,317	25 bp	reverse	unsuccessful	monocots	this study

note: [1] italicized numbers indicate base positions that partly lie within introns (GT motive of intron start).

Table 9. Primers tested for phosphoribulokinase (*PRK*). Highlighted primers performed best and were selected for PCR or sequencing. Position of primers was determined from the assembled RefSeq genome of *Oryza sativa*. bp = base pairs. CDS = coding sequence. Chr = chromosome.

name	sequence (5'–3')	CDS position (bp)	Chr position (bp)	length	direction	use/success	specificity	reference
primers initially tested								
prk488f [1]	AAYGAYTTTGAYCTYATGTATGARCARGT	exon 1, 394–422	29,589,269–29,589,297	29 bp	forward	unsuccessful	angiosperms	Lewis & Doyle 2002
prk596f (prk663f)	GAYTTCAGYATYTAYTTRGACAT	exon 2, 574–596	29,589,742–29,589,764	23 bp	forward	PCR/sequencing	angiosperms	D. Springate, RBG Kew, unpublished
prK973r (prk1040r)	TCTRTCAAATTGYCCRTCCATYTC	exon 5, 973–996	29,590,630–29,590,653	24 bp	reverse	PCR/sequencing	angiosperms	D. Springate, RBG Kew, unpublished
prk1167r	ATGGTYTGRAANARACCNGTNCCRTTGTTGC	exon 5, 1,100–1,130	29,590,757–29,590,787	31 bp	reverse	PCR/sequencing	angiosperms	Lewis & Doyle 2002
primers subsequently tested								
prk621f	TCAGCAATGAGGTTAAATTTGCATGG	exon 2, 596–621	29,589,764–29,589,789	26 bp	forward	PCR	bromeliads	this study; modified from "prk622f"
prk622f	CAGCAATGAGGTTAAATTTGCATGGA	exon 2, 597–622	29,589,765–29,589,790	26 bp	forward	PCR/sequencing	bromeliads	Schulte, Barfuss & Zizka 2009
prk630f [2]	AAATTTGCATGGAAAATTCAGGTC	exon 2, 610-630 (*–633*)	29,589,778–29,589,801	24 bp	forward	sequencing	bromeliads	this study
prk734f (prk735f) [2,3]	CTGCAGATCCGCAGAAGAAATATGC	exon 4, (*710*–) 716–734	29,590,137–29,590,161	25 bp	forward	sequencing	bromeliads	Schulte, Barfuss & Zizka 2009
prk734f-2 [2,4]	CCGCAGATCCGCAGAAGAAATTTTC	exon 4, (*710*–) 716–734	29,590,137–29,590,161	25 bp	forward	sequencing	Brocchinia	this study; modified from "prk734f"
prk889r	GGGTATGAGCATGTCAATTTCCTCCC	exon 4, 889–914	29,590,316–29,590,341	26 bp	reverse	sequencing	bromeliads	Schulte, Barfuss & Zizka 2009
prk890r	GGGTATGAGCATGTCAATTTCCTCC	exon 4, 890–914	29,590,317–29,590,341	25 bp	reverse	sequencing	bromeliads	this study; modified from "prk889r"
prk1057r	CTTCAGCATTTGTTGTGTCACCTC	exon 5, 1,057–1,080	29,590,714–29,590,737	24 bp	reverse	sequencing	bromeliads	this study
prk1069r	GAAAATCTGCRTGCTTCAGCATTTG	exon 5, 1,069–1,093	29,590,726–29,590,750	25 bp	reverse	PCR/sequencing	bromeliads	Schulte, Barfuss & Zizka 2009
prk1069r-2	GGAAAATCTGCRTGCTTCAGCATTTG	exon 5, 1,069–1,094	29,590,726–29,590,750	26 bp	reverse	PCR	bromeliads	this study; modified from "prk1069r"

notes: [1] unlike given in Lewis & Doyle (2002) primer position of "prk488f" is in exon 1 but not in exon 3.
[2] italicized numbers indicate base positions that partly lie within introns.
[3] position of "prk735f" was initially wrongly determined (Schulte & al, 2009) and therefor corrected from "prk734f".
[4] for the genus *Brocchinia* primer "prk734f" had to be modified because of mismatches that give problems in sequencing.

Table 10. Primers used/tested for phytochrome C (*PHYC*). Highlighted primers performed best and were selected for PCR or sequencing. Position of primers was determined from the assembled RefSeq genome of *Oryza sativa*. bp = base pairs. CDS = coding sequence. Chr = chromosome.

name	sequence (5'–3')	CDS position (bp)	Chr position (bp)	length	direction	use	specificity	reference
primers used initially for primer design								
PHYupstream1	TCWGGNAARCCNTTYTAYGC	exon 1, 484–503	31,768,662–31,768,681	20 bp	forward	primer design	angiosperms	Mathews & Donoghue 1999
PHYupstream2	CCITTYTAYGSIATHYTICAYMG	exon 1, 493–515	31,768,671–31,768,693	23 bp	forward	primer design	angiosperms	Mathews & Donoghue 1999
PhyCdownstream	GRATKGCATCCATYTCMAYRTC	exon 1, 1,705–1,726	31,769,883–31,769,904	22 bp	reverse	primer design	angiosperms	Mathews & Donoghue 1999
primers subsequently tested								
phyc503f-mo	TCVGGGAAGCCSTTYTAYGC	exon 1, 484–503	31,768,662–31,768,681	20 bp	forward	PCR/sequencing	monocots	Russell & al., 2010b, modified from "PHYupstream1"
phyc515f-mo	AAGCCCTTYTACGCVATMATGCACCG	exon 1, 490–515	31,768,668–31,768,693	26 bp	forward	PCR/sequencing	monocots	this study, modified from "PHYupstream2"
phyc515f-br	AAGCCCTTYTACGCTATCCTGCACCG	exon 1, 490–515	31,768,668–31,768,693	26 bp	forward	PCR	bromeliads	this study, modified from "PHYupstream2"
phyc524f-br	GCTATCCTGCACCGGATCGAYGT	exon 1, 502–524	31,768,680–31,768,702	23 bp	forward	sequencing	bromeliads	this study
phyc974f-mo	GCTCCTCAYGGCTGYCAYGCTCA	exon 1, 952–974	31,769,130–31,769,152	23 bp	forward	sequencing	some monocots: bromeliads, orchids	this study
phyc974f-br	GCTCCTCACGGCTGCCACGCTCA	exon 1, 952–974	31,769,130–31,769,152	23 bp	forward	sequencing	bromeliads	this study
phyc991f-mo	CCAYGCTCARTAYATGGCTAATATGG	exon 1, 966–991	31,769,144–31,769,169	26 bp	forward	sequencing	some monocots: bromeliads, orchids	this study
phyc1145r-mo	CCTGMARCARGAACTCACAAGCATATC	exon 1, 1,145–1,171	31,769,323–31,769,349	27 bp	reverse	sequencing	some monocots: bromeliads, orchids	Russell & al., 2010b
phyc1145r2-mo	CAACAGGAACTCACAAGCATATC	exon 1, 1,145–1,167	31,769,323–31,769,345	23 bp	reverse	sequencing	some monocots: bromeliads, orchids	this study; modified from "phyc1145r-mo"
phyc1210r-mo	GGATATGCTTCTCCTTTGYTTGAGC	exon 1, 1,210–1,234	31,769,388–31,769,412	25 bp	reverse	sequencing	some monocots: bromeliads, orchids	this study
phyc1690r-br	TCAACATCTTCCCAYGGGAGGCT	exon 1, 1,690–1,712	31,769,868–31,769,890	23 bp	reverse	sequencing	bromeliads	this study
phyc1699r-mo	ATWGCATCCATTTCAACATCYTCCCA	exon 1, 1,699–1,724	31,769,877–31,769,902	26 bp	reverse	PCR/sequencing	monocots	this study
phyc1699r-br	ATWGCATCCATTTCAACATCTTCCCA	exon 1, 1,699–1,724	31,769,877–31,769,902	26 bp	reverse	PCR	bromeliads	this study
phyc1705r-mo	GRATWGCATCCATYTCAACATC	exon 1, 1,705–1,726	31,769,883–31,769,904	22 bp	reverse	PCR/sequencing	monocots	Russell & al., 2010b, modified from "PhyCdownstream"

Table 11. Attributes of analyzed matrices and parsimony scores of equally most parsimonious trees after analysis using PAUP*. ITS nrDNA was analysed with (incl.) and without (excl.) the problematic region of ITS1. Data of plastid DNA was taken from Barfuss & al., (2005). The sequence length is given for individual markers only. bp = base pairs, var. char. = variable characters, PUIC = parsimony uninformative characters, PIC = parsimony informative characters, CI = consistency index, RI = retention index, RC = rescaled consistency index, HI = homoplasy index.

marker	no. of taxa studied	amplicon lenght [bp]	no. of ch.	no. of constant ch.	no. of variable ch.	no. of PUIC.	no. of PICs	no. of trees	tree length	CI	RI	RC	HI
ITS nrDNA (incl.)	111	884–1,017	1,291	899 (69.6%)	392 (30.4%)	173 (13.4%)	219 (17.0%)	>10,000	936	0.590	0.748	0.441	0.410
ITS nrDNA (excl.)	111	884–1,017	1,057	788 (74.5%)	269 (25.5%)	120 (11.4%)	149 (14.1%)	>10,000	634	0.588	0.779	0.458	0.412
MS	13	659–707	710	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.
RPB2	8	520–522	523	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.
PGIC	2	983	983	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.
NIA	72	649–800	1,022	734 (71.8%)	288 (28.2%)	164 (16.1%)	124 (12.1%)	>10,000	527	0.691	0.784	0.542	0.309
XDH	64	1,110	1,110	860 (77.5%)	250 (22.5%)	138 (12.4%)	112 (10.1%)	>10,000	369	0.743	0.740	0.549	0.257
PRK	444	831–1,692	3291	2,554 (77.6%)	737 (22.4 %)	203 (6.2%)	534 (16.2%)	>10,000	2,232	0.478	0.870	0.416	0.522
PHYC	444	1159–1,192	1,228	743 (60.5%)	485 (39.5%)	116 (9.4%)	369 (30.1%)	>10,000	1,398	0.473	0.867	0.410	0.527
plastid DNA	120	∑ of 7 markers	6,277	5,294 (84,3 %)	983 (15,7 %)	440 (7.0%)	543 (8.7%)	>10,000	1,713	0.654	0.830	0.543	0.346

Amplification, sequencing, and cloning of low-copy nuclear DNA markers

Gradient PCR conditions were optimized with either a Mastercycler gradient (Eppendorf) or a Veriti 96-Well Thermal Cycler (Applied Biosystems, Life Technologies) using their gradient function or independent temperature blocks, respectively. Gradient PCR was usually done in two successive steps, (1) running gradients for the annealing temperature, and (2) running gradients for the extension temperature.

The first step is necessary to find the best annealing temperature for a given primer pair, which can differ greatly from calculated melting temperatures using different programs for oligonucleotides (e.g., NetPrimer; http://www.premierbiosoft.com/netprimer/index.html). General PCR conditions for the first step were: 1× 95°C for 2 min; 35× 95°C for 30 s, X°C for 30 s, 72°C for Y min; 1× 72°C for 7 min; and 4°C for ∞; where X stands for the annealing temperature variation and Y for the extension time used. The latter was dependent on the expected fragment length and ranged between one and three minutes according to the general rule that Taq amplifies at a rate of 1 kb per minute. Usually two rounds of annealing temperature gradients were performed, initially with a wide temperature variation of 48–68°C and later a narrower range determined based on the results of the first round.

The second step was done because the extension temperature depends not only on the working optimum of Taq polymerase but also on the base composition (GC content) in certain areas of the amplified region (Su & al., 1996). AT-rich sections might need a reduced extension temperature, since these segments start to denature sometimes even below 72°C and Taq polymerase is not able to function. The online program POLAND (Steger, 1994; http://www.biophys.uni-duesseldorf.de/html/local/POLAND/poland.html) can be used to check for 50% temperature probabilities of each base in a given sequence, which is a theoretical calculation using different formulas for how strongly bases are bound to each other. This presents a problem with this approach because at least one sequence of the targeted region for a given study group is required a priori to conduct this analysis. The general PCR conditions for the second step were: 1× 95°C for 2 min; 35× 95°C for 30 s, X°C for 30 s, Z°C for Y min; 1× Z°C for 7 min; and 4°C for ∞; where Z refers to a temperature variation of 60–72°C. Usually one gradient run was sufficient to verify whether a denaturing temperature of 72°C is too high.

A third gradient step to determine the denaturing temperature was necessary only for ITS nrDNA, because Taq is not able to pass through GC-rich regions, which are still bound at 72°C because they were not denatured at 95°C completely. If necessary, further PCR optimization steps were undertaken and/or specific PCR conditions established for each marker (Table 2). Occasionally other parameters for markers such as two-step PCR, inclusion of a touchdown cycle, or an increase of the extension time by 5 s at later cycles has been shown to produce greater amounts of PCR product. Each gradient PCR was done in 0.2 mL 12-strip PCR tubes (or 96-well PCR plates) using 10 µL reactions including 9 µL 1.1× ReddyMix PCR Master Mix containing 2.5 mM $MgCl_2$ (AB-0619; Thermofisher, ABgene), 0.4 µM of each primer (0.2 µL at 20 µM = 20 pmol/µL), 4% (0.4 µL) dimethysulfoxide (DMSO), and 0.2 µL (approximately 25 ng/µL) template DNA.

Amplifications of each marker using optimized conditions were usually carried out one a Mastercycler gradient in 25 µL reactions using 22.5 µL 1.1× ReddyMix PCR Master Mix containing 2.5 mM $MgCl_2$, 0.5 µL (0.4 µM) of each primer at 20 µM, 1 µL (4%) DMSO, and 0.5 µL template DNA. Volumes were scaled up proportionally when more PCR product was necessary to perform cycle sequencing with additional primers. When single bands were present on agarose

gels, PCR products were purified either with a 1:2 mixture of exonuclease I (20 units/µL; Fermentas) and alkaline phosphatase (either shrimp or FastAP thermosensitive alkaline phosphatase, 1 unit/µL; Fermentas) or ExoSAP-IT (Amersham, GE Healthcare) to degrade single stranded DNA fragments and dNTPs (Werle & al., 1994); 2.5 µL of the enzyme mixture (scaled up proportionally for higher volumes) was added to each 25 µL PCR reaction and incubated at 37°C for 45 min, followed by deactivating the enzymes at 85°C for 15 min.

Cycle sequencing reactions were usually performed on a 96-Well GeneAmp PCR System 9700 (Applied Biosystems, Life Technologies) using a modified reaction protocol: 0.5 µL BigDye Terminator v3.1 (Applied Biosystems, Life Technologies), 1 µL (0.4 µM) primer at 4 µM, 1.75 µL 5× sequencing buffer, 3–6.75 µL purified PCR product, and 0–3.75 µL PCR-grade water. The general temperature profile (except for ITS nrDNA) was: 1× 96°C for 1 min; 35× 96°C for 10 s, 50°C for 5 s; 60°C for 3 min; 4°C for ∞. Modifications to the original profile are increased number of cycles (orig. 25 cycles) and reduced extension time (orig. 4 min). Cycle sequencing products were purified by gel filtration using MultiScreen filter plates (MAHVN4550; Milipore) and the cross-linked dextran gel Sephadex G-50 Superfine or Fine (GE Healthcare) according to the Millipore protocol (Tech Note TN053) with few modifications (more details provided upon request from the first author). Sequences were run on either a 16-capillary sequencer (3130*x*/ Genetic Analyzer, Applied Biosystems, Life Technologies) or a 48-capillary sequencer (3730 DNA Analyzer; Applied Biosystems, Life Technologies) following manufacturer's instructions.

When cloning was required, the proof-reading Phusion High-Fidelity DNA polymerase (F-530S; Finnzymes) was used to reduce the accumulation of polymerase errors in the cloned sequences and the formation of artificial chimeric PCR products. PCR reactions were 50 µL, including 34.7 µL purified water, 10 µL 5× Phusion High Fidelity PCR buffer, 0.8 µL $MgCl_2$ at a concentration of 50 mM, 1 µL dNTPs at 10 mM each, 0.5 µL Phusion DNA polymerase (2U/µL), 1 µL of each primer at 20 mM, and 1 µL template DNA. Thermocycling was performed according to manufacturer's instructions with slight modifications to fit the optimize PCR conditions for each marker. Amplified DNA was verified and purified on 1% TAE agarose gels using the Invisorb Spin DNA Extraction Kit (Invitek) following the manufacturer's instructions. Since the proof reading DNA polymerase is not able to create 3' A-overhangs, which is necessary to proceed with standard TA cloning, purified PCR products were incubated at 72°C for 20 min using a mixture of 0.5 µL dATPs at 10 mM, 2.5 µL 10× Taq PCR buffer containing $(NH_4)_2SO_4$, 0.1 µL Taq polymerase (5U/µL), and 1.5 µL $MgCl_2$ at 25 mM per 25 µL purified PCR product (all products from Fermentas). Cloning was performed using the pGEM-T Easy Vector System (Promega) following manufacturer's instructions. Successfully transformed colonies were suspended in 1× TE buffer, denatured for 10 min at 95°C, placed on ice, centrifuged to collect cell debris, and used as a template for PCR. Subsequent amplification and sequencing were performed as described above.

Amplification and sequencing of ITS nrDNA

PCR of the ITS nrDNA (Figure 1, A) was initially attempted using the universal primers "ITS2", "ITS3", "ITS4" and "ITS5" (White & al., 1990; Baldwin & al., 1995) and the angiosperm-specific primers "17SE" and "26SE" (Sun & al., 1994; Douzery & al., 1999). No useful PCR products were obtained at the first step of gradient PCR using standard reactions. Therefore the new angiosperm-specific PCR primers "ITS18Sf", "ITS5.8Sf", "ITS5.8Sr", and "ITS26Sr" with a significantly higher annealing temperature were employed (Gruenstaeudl & al., 2009). These primers have been developed using large-unit ribosomal sequences of angiosperms from GenBank plus a

sequences of Bromeliaceae. The new internal 5.8S nrDNA primers, “ITS5.8Sf” and “ITS5.8Sr”, were used because “ITS3” and “ITS2” of White & al. (1990) do not produce overlapping reads and have a low annealing temperature. For further PCR trials, standard reactions were altered by adding DMSO in final concentrations of up to 10% and/or betaine up to 1 M; these additives are thought to aid in amplification by reducing GC binding during extension. Betaine had no obvious effect, but DMSO at a final concentration of 3% produced a PCR product of the expected length in at least some samples. Therefore, in a final optimization step a gradient run for the denaturing temperature was performed using a temperature range of 94–99°C. Already at 96°C more samples showed the expected PCR product and at a denaturing temperature of 99°C consistently all samples had comparatively strong bands. Additionally an extension time twice as high as the general rule (1 kb/m) increased the amount significantly. The optimized PCR conditions are given in Table 2.

For the initial phase of cycle sequencing for ITS nrDNA, all four newly generated primers and the standard reaction protocol and PCR conditions were used. Two problems in these sequences were detected. Firstly electropherograms had n-1 patterns because of high primer melting temperatures (around 70 °C), which was solved by using the primers from Blattner (1999) and/or several newly designed sequencing primers that have annealing temperatures around 65°C. The second problem was that only internal primers sequencing towards the 26S nrDNA gave complete reads. In all other cases signal intensity dropped and was mostly completely lost. In such cases, the dGTP BigDye Terminator v3.0 Cycle Sequencing Kit (Applied Biosystems, Life Technologies) procedure has been modified. To be able to run this chemistry on the capillary sequencers it was mixed in a ratio of 1:4 (dGTP v3.0:v3.1) with the BigDye Terminator v3.1 to avoid strong banding compression patterns that are visible in capillary electrophoresis by using the dGTP BigDye Terminator v3.0 alone. Also the effect of DMSO and betaine was tested. The general sequencing protocol was modified by using 1 µL per reaction of the BigDye Terminator mix, 1 µL (0.4 µM) primer at 4 µM, 1 µL 5× sequencing buffer, 1.6 µL (0.8 M) betaine at 5 M, 0.4 µL (4%) DMSO, 4 µL purified PCR product, and 1 µL PCR-grade water. Cycling conditions were changed to: 1× 96°C for 1 min, 99°C for 30 s, and 60°C for 3 min; 35× 96°C for 20 s, 60°C for 3 min; and 4°C for ∞. No annealing step was necessary since all primers used for sequencing have annealing temperatures higher than 60°C. Details of all PCR and sequencing primers tested and finally used for ITS nrDNA are given in Table 3.

Analyses of nuclear DNA sequences

Raw sequences were initially analyzed and edited using the Sequencing Analysis Software v5.3 (Applied Biosystems, Life Technologies). Forward and reverse DNA strands from both PCR and/or internal sequencing primers were assembled with the SeqMan Pro module of the Lasergene v8.1 software package (DNASTAR). Contigs were edited and allelic consensus sequences were exported as text file in fasta format. Fasta files were pooled and aligned with MUSCLE v3.8 (Edgar, 2004a, b) leaving default parameters unchanged and then adjusted by eye with BioEdit v7.0.5 (Hall, 1999) following the guidelines of Kelchner (2000) and Borsch & Quandt (2009). In polymorphic DNA regions containing numerous insertion/deletion (indel) events related taxa were aligned first. Later these blocks of aligned taxa were aligned using the profile alignment function of MUSCLE and adjusted by eye using BioEdit.

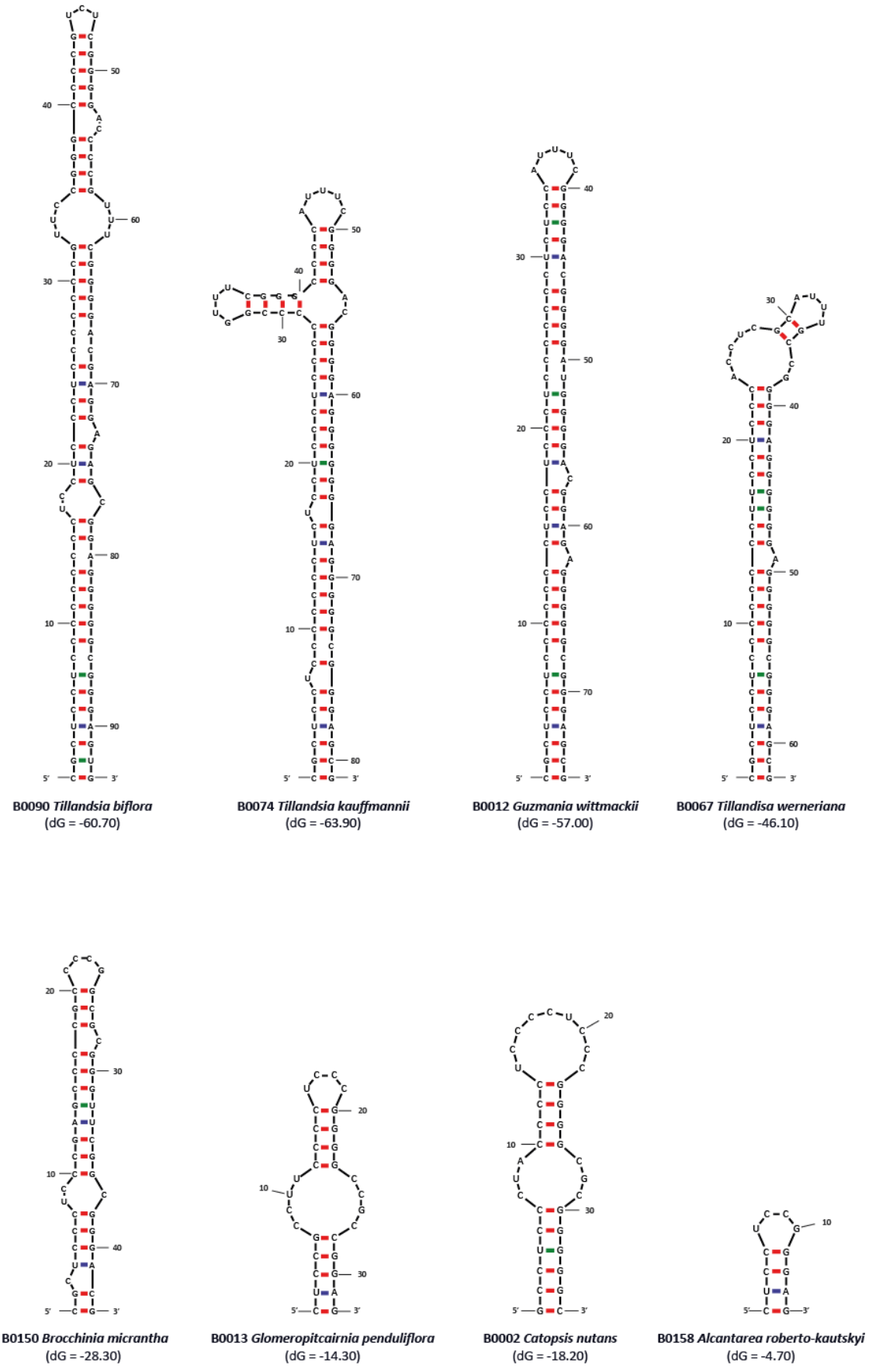


Figure 2. Secondary structure (hairpins) for eight selected taxa of Tillandsioideae of the same problematic region of the ITS1 obtained from RNA folding on the mfold web sever.

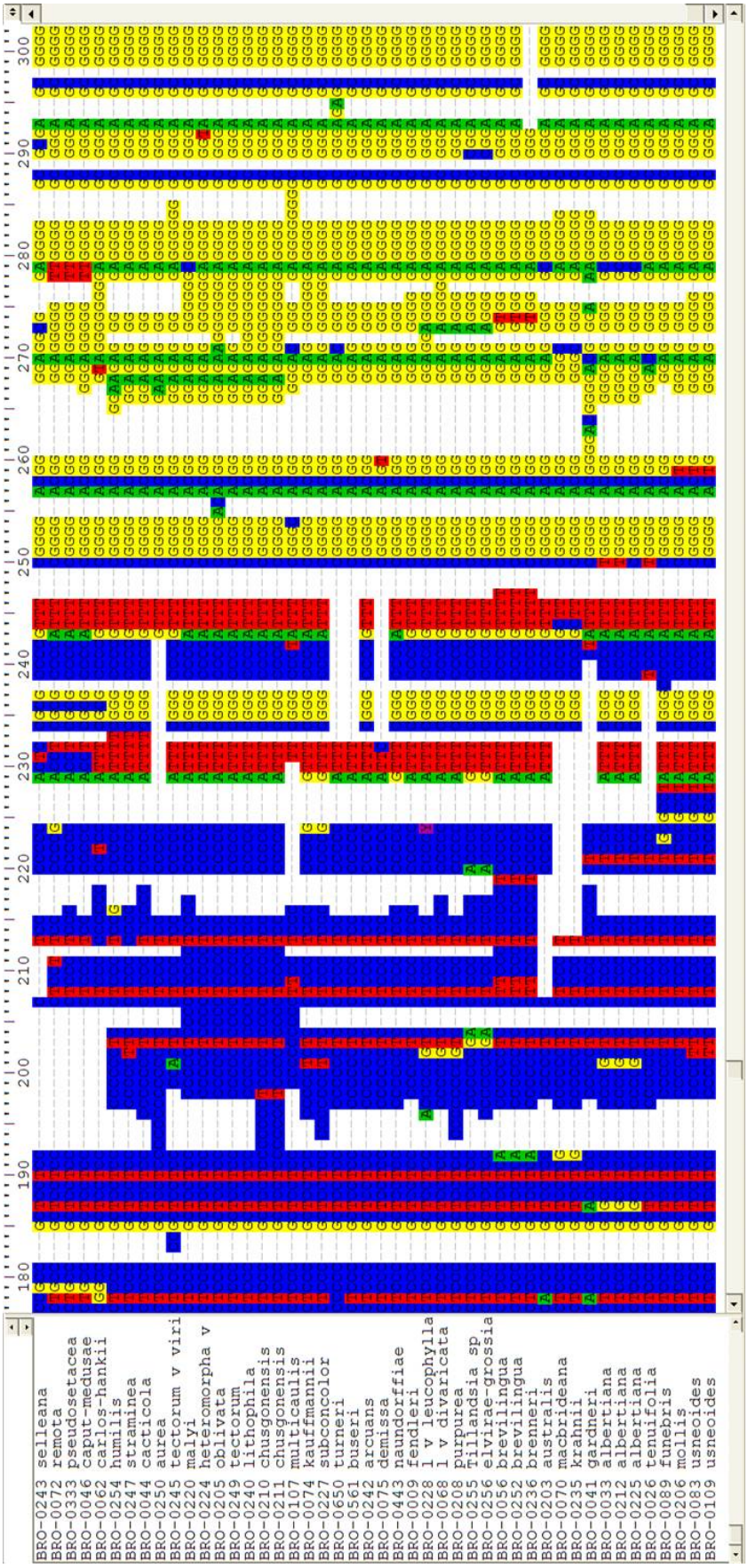


Figure 3. Alignment of a section of the ITS1 region showing the problematic region that has an extremely stable secondary structure, which gives problems in PCR and sequencing.

The Alignment of ITS nrDNA was guided by their secondary structures (Figures 2, 3). Stem-loop regions (hairpins) were identified either visually or on the mfold web server (Zuker, 2003). A certain GC rich region within ITS1 could not be unambiguously aligned (Figure 3). Aligned ITS nrDNA sequences were checked for the presence of the conserved angiosperm motifs -GGCRY-[4–7 N]-GYGYCAAGGAA- in ITS1 (Liu & Schardl, 1994), -GAATTGCAGAATCC- in the 5.8S nrDNA region (Jobes & Thien, 1997), and the conserved (C1–C6) and variable (V1–V6) domains in ITS2 (Hershkovitz & Zimmer, 1996) to exclude pseudogenes. Alignments of low-copy nuclear DNA markers were adjusted by checking the reading frame, by determining the angiosperm-specific intron-flanking GT-AG motive in intron regions, and by using annotated genes of the assembled RefSeq genome of *Oryza sativa* and other annotated sequences from GenBank. Aligned matrices can be obtained from the first author upon request.

Since PCR products can contain two or more alleles and were mostly sequenced directly in this study, heterozygous individuals contain two types of polymorphisms: (1) single nucleotide polymorphisms (SNPs) that are visible as double peaks at certain positions, and (2) length polymorphisms caused by insertion/deletion (indels) events, which result in polymorphisms at every subsequent position in the electropherogram. Unambiguous SNPs were coded using the IUPAC-IUB symbols for nucleotide nomenclature. Indels made assembly of contigs difficult. To be able to assemble such contigs, the type of indel was identified by visual inspection and usually the shorter allele was ignored and manually overwritten by the sequence of the longer. Assembled contigs were edited with special care to correct for handling mistakes and the initially ignored sequence of the shorter allele was checked not to miss any mutations. If two or more indels between priming sites were observed, editing was highly problematic and error-prone; in such cases, these individuals were excluded or the PCR products cloned and sequenced.

Nuclear DNA alignments were inspected prior to phylogenetic analyses for the impact of SNPs and indels within allelic consensus sequences of a given individual (Jabaily & Sytsma, 2010). The presence of similar alleles in more distantly related taxa may indicate possible hybridization events including reticulations and introgressions, polyploidization, and occurrence of incomplete lineage sorting. When no significant conflict was detected (homoplasious SNPs within accessions of distantly related taxa were ignored), sequences were included and differences were assumed to be most likely due to allelic variation within a single species or within a species complex. Otherwise individuals were excluded or sequenced after cloning. Sequences resulting from PCR-mediated, artificial recombinations of alleles were sometimes observed and excluded.

PAUP* version 4.0b10 (Swofford, 2003) has been used for all analyses using maximum-parsimony with unordered and equally weighted characters. A two-step heuristic search method was used: (1) 1,000 random sequence additions, TBR branch swapping holding 10 trees each step, and saving 10 shortest trees per replicate; (2) trees obtained from the first analyses were taken as starting trees and heuristic searches were conducted until all or a maximum of 10,000 shortest trees were saved, but the tree search was allowed to continue to check all input trees for possible shorter ones (swapping to completion). Gaps were treated as missing characters, and no gap coding procedure was applied because of the many indels within accessions that were ignored within allelic consensus sequences. The matrices including all characters were analyzed for five nuclear DNA markers (ITS nrDNA, *NIA*, *XDH*, *PRK*, *PHYC*) and for ITS nrDNA alone in a matrix excluding the difficult region of ITS1 (Figures 2,3), since the alignment was ambiguous for that region and many gaps had to be introduced. For compari-

son, the plastid matrix of Barfuss et al. (2005) was reanalyzed with the same phylogenetic methods, but the super-outgroup *Stegolepis* (Rapateaceae) has been excluded to be comparable with results of the other loci. Statistics for each marker are given in Table 11.

Support was estimated with the bootstrap (Felsenstein, 1985). Bootstrap percentages (BP) were calculated using 1,000 replicates, TBR branch swapping, and simple sequence addition, holding 10 trees each step and saving 100 shortest trees per bootstrap replicate.

Results

Direct sequencing of the eight nuclear DNA markers was possible in nearly all accessions. Comparisons of amplified fractions of nuclear DNA markers to the assembled RefSeq genome of *Oryza sativa* showed that *MS*, *RPB2*, *XDH*, and *PHYC* are single-copy in rice (Table 1). Although present in multiple copies in *Oryza sativa*, blast of *PGIC*, *NIA*, and *PRK* fragments produced in this study showed greatest similarity to the same single paralog.

Nuclear ribosomal internal transcribed spacer (ITS nrDNA)

Previously published protocols and PCR primers did not consistently produce PCR products, indicating that either the primers did not match the target or the protocol was inappropriate. The newly developed primers with a significantly higher annealing temperature and higher DMSO concentrations produced PCR products in few samples if the standard denaturing temperature was used. Therefore high strong secondary structure are likely present in bromeliad ITS nrDNA, which is insufficiently denatured at 95°C to allow successful amplification at 72°C (Figure 2). At higher denaturing temperatures PCR bands of the expected length began to appear but yields were variable; at 99°C, all samples amplified consistently. As would be suspected from high GC content (Figure 3), cycle sequencing was also problematic. By altering the sequencing chemistry it was possible to get full ITS nrDNA sequences for all included accessions, although a three-step loss of signal still occurred. Some taxa gave problems even with this modified protocol and were finally excluded.

With the primers finally used in this study, a standard ITS nrDNA sequence was composed of 123 bp of 18S nrDNA, ITS1 (of variable length), 164 bp for 5.8S nrDNA, ITS2 (of variable length), and 139 bp of 26S nrDNA. Fragment size ranged from 884–1,017 bp (in *Tillandsia biflora* and *Vriesea scalaris*, respectively). No pseudogenes were found, although slight changes to the published motifs of the conserved and variable domains described previously for ITS2 were found. The final matrices included 1,291 (including all characters) and 1,057 (excluding the problematic ITS1 region) positions, respectively. Other statistics are given in Table 11.

For ITS nrDNA strict consensus trees (Figure 4), bootstrap consensus trees (Figure 5) and phylograms (Figure 6) are shown, resulting from analyses of the total matrix as well as of a matrix excluding the problematic ITS1 region. Although several clades of related species received bootstrap support, backbone relationships are insufficiently resolved. Each accession is different from the rest (i.e. long terminal branches) but synapomorphies are few (i.e. short internal branches), as can be seen in one of the individual parsimony trees (Figure 6, Table 11).

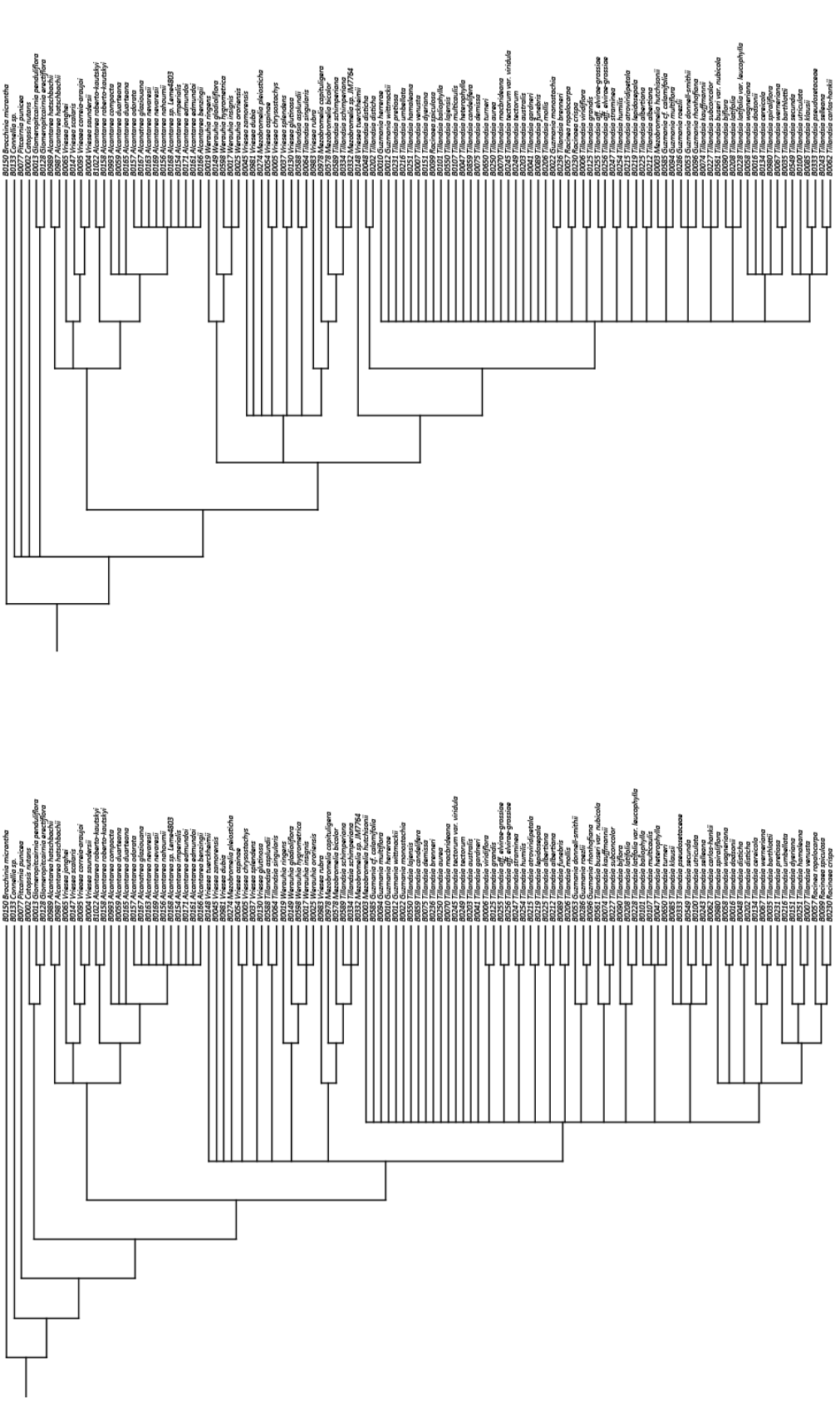


Figure 4. Strict consensus trees of the ITS nrDNA: analysis of the complete DNA sequence matrix (A), analysis excluding the problematic region of ITS 1 (B).

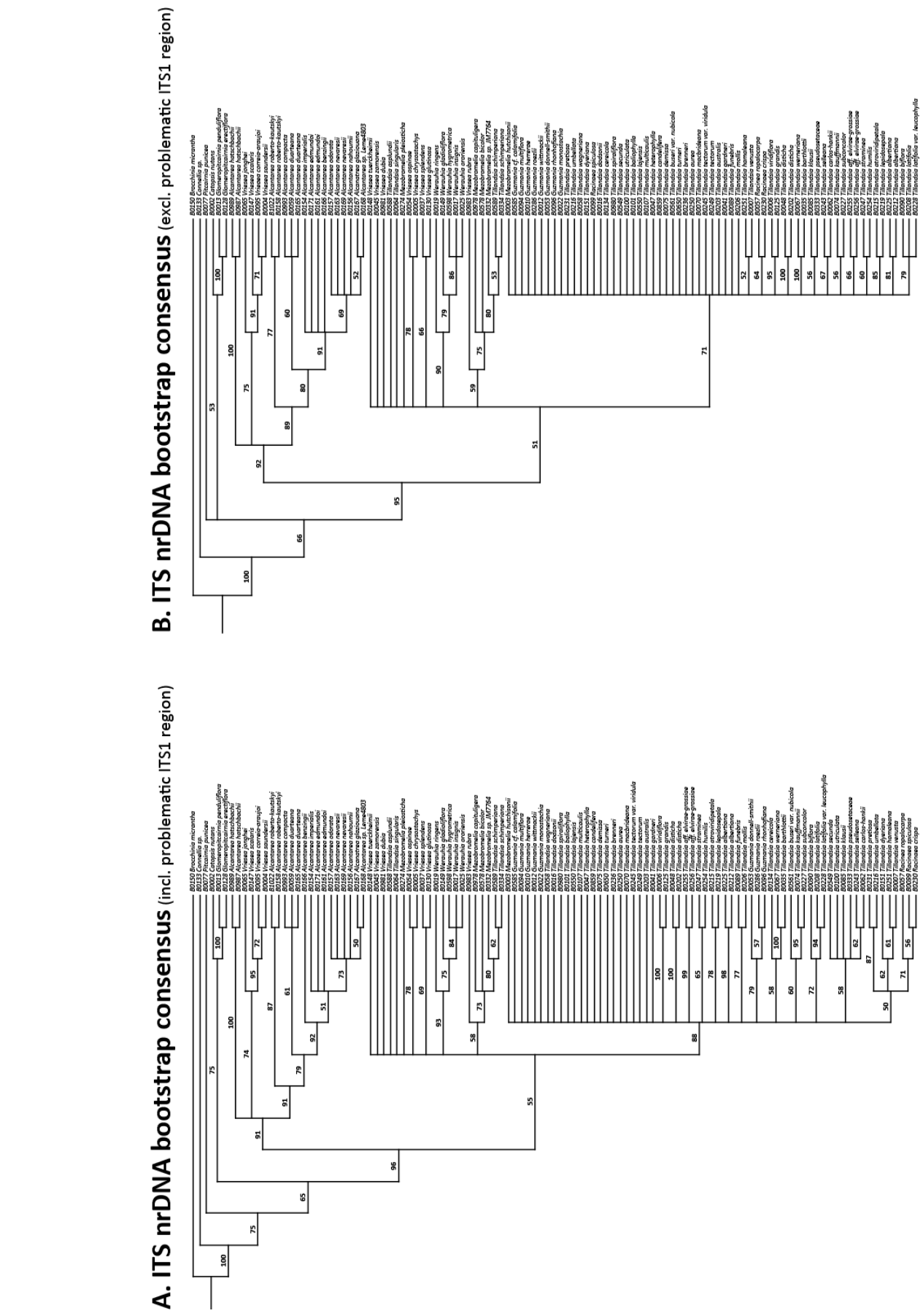


Figure 5. Bootstrap consensus trees of the ITS nrDNA: analysis of the complete DNA sequence matrix (A), analysis excluding the problematic region of ITS 1 (B).

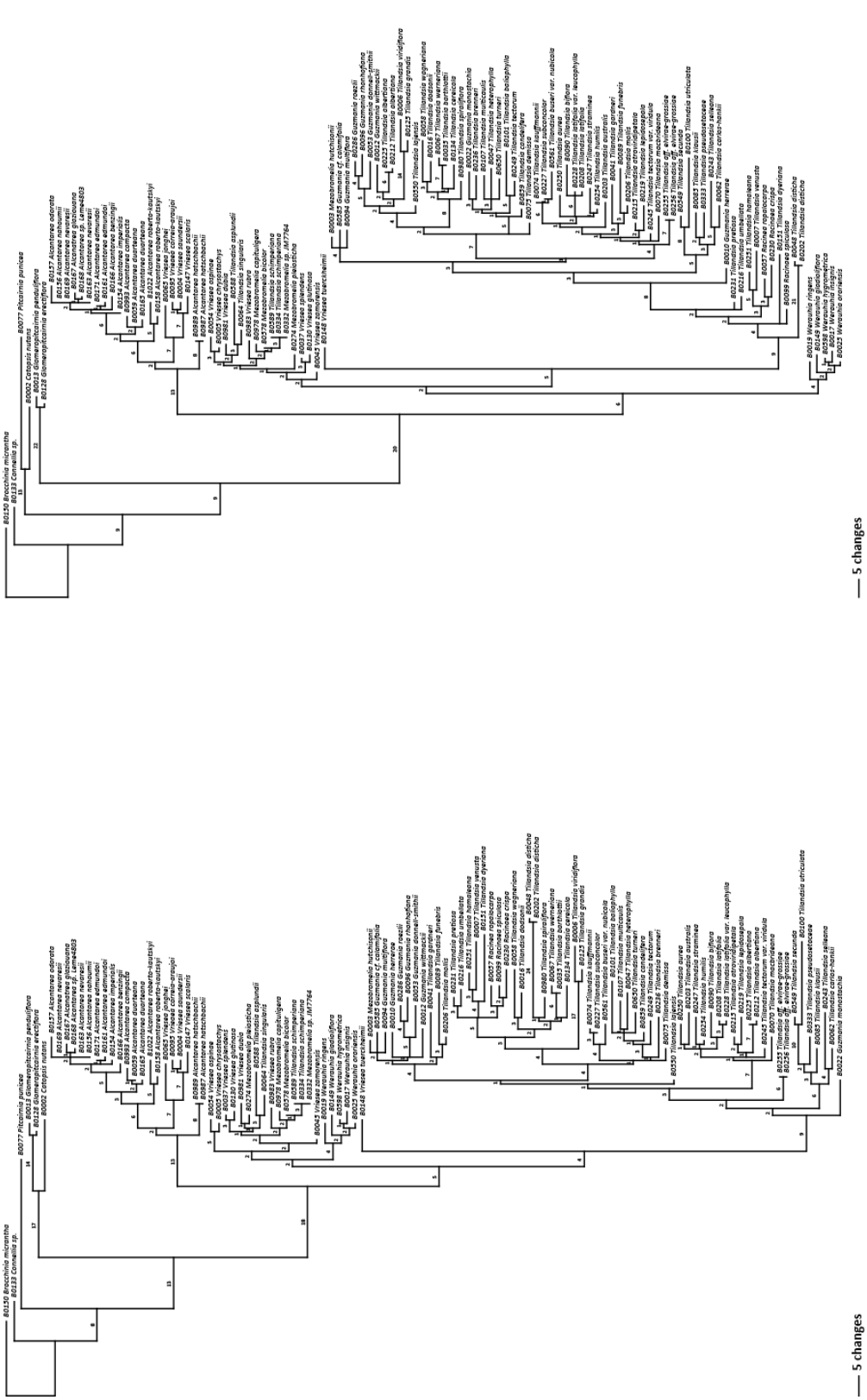


Figure 6. Phylograms selected randomly from 10,000 trees saved during maximum parsimony analyses of the ITS nrDNA: analysis of the complete DNA sequence matrix (A), analysis excluding the problematic region of ITS 1 (B).

Malate synthase (MS) and RNA polymerase II, beta subunit (RPB2)

MS (Figure 1, B) was initially attempted using the primers "ms400f", "ms526f", "ms943r" and "ms1488r" of Lewis & Doyle (2001) and the modified or new primers "ms356f" and "ms1408r" (D. Springate, RBG Kew, London, unpublished) in all possible combinations. Since no amplifications were obtained, new primers were designed based on *MS* sequences obtained from GenBank. Three forward and three reverse primers were developed covering a region from partial exon 1 to partial exon 4. The position of internal primers was designed to amplify three overlapping fragments that might be assembled as one piece. New primers were tested in all possible combinations, but only amplification of the fragment containing intron 2 using the primers "ms428f" and "ms960r" was successful and further optimized (Table 2). PCR primers were used as sequencing primers. Details of all primers tested for *MS* are given in Table 4. *RPB2* (Figure 1, C) was tested using primers "P6F", "P7R", "P7F", "10R", "P10F", and "11aR" (Table 5) of Denton & al. (1998) in all possible combinations for a region covering partial exon 11 to partial exon 24. Only the primer combination "P10F"/"P11aR" successfully amplified a fragment that contains intron 23. PCR conditions were further optimized (Table 2) and the product finally sequenced using PCR primers.

Both markers were tested for two small sets from *Tillandsia* species complexes (*T. tectorum*, *T. plumosa*) with *Mezobromelia hutchisonii* as outgroup. The amplified fragment of *MS* (659–707 bp) covered a region from partial exon 2 (279–282 bp) to partial exon 3 (261 bp) (Figure 1, B), that of *RPB2* (520–522 bp) a region from partial exon 23 (148 bp), to partial exon 24 (134 bp) (Figure 1, C). Both markers displayed indel variation, but only few substitutions. No phylogenetic analysis was conducted due to the limited number of taxa and sequence variability.

Glucose-6-phosphate isomerase, cytosolic (PGIC)

Amplification of *PGIC* (Figure 1, D) was attempted using primers of Ford & al. (2006) in different combinations. Primer combination "AA11F"/"AA16R" for a fragment covering parts of exon 11 to exon 16, respectively, was the only successful one. PCR primers were used as sequencing primers. Optimized PCR conditions and primer details are given in Table 2 and Table 6, respectively.

PGIC was currently tested only for two species, one each from *Catopsis* and *Alcantarea*. The amplified fragment (983 bp) covered a region from partial exon 11 (17 bp) to partial exon 16 (6 bp) (Figure 1, D). Two 1-bp insertions and 53 substitutions were observed. No phylogenetic analysis was performed, due to limited sampling.

Nitrate reductase 1, [NADH] (NIA)

NIA (Figure 1, E) was initially tested using the primers combinations "NIA2F"/"NIA2R" (targeting intron 2) and "NIA3F"/"NIA3R" (targeting intron 3) of Howarth & Baum (2002). Amplification of intron 2 failed and the primers for intron 3 only amplified a ca. 200 bp fragment. Therefore new primers were designed to cover a region from partial exon 1 to partial exon 3. Several primer combinations have been tested, but only the combination "nia410f"/"nia1042r" covering a part of exon 1 and the complete intron 1 gave satisfactory results and was therefore optimized further (Table 2). PCR primers were used as sequencing primers. Primer details are given in Table 7.

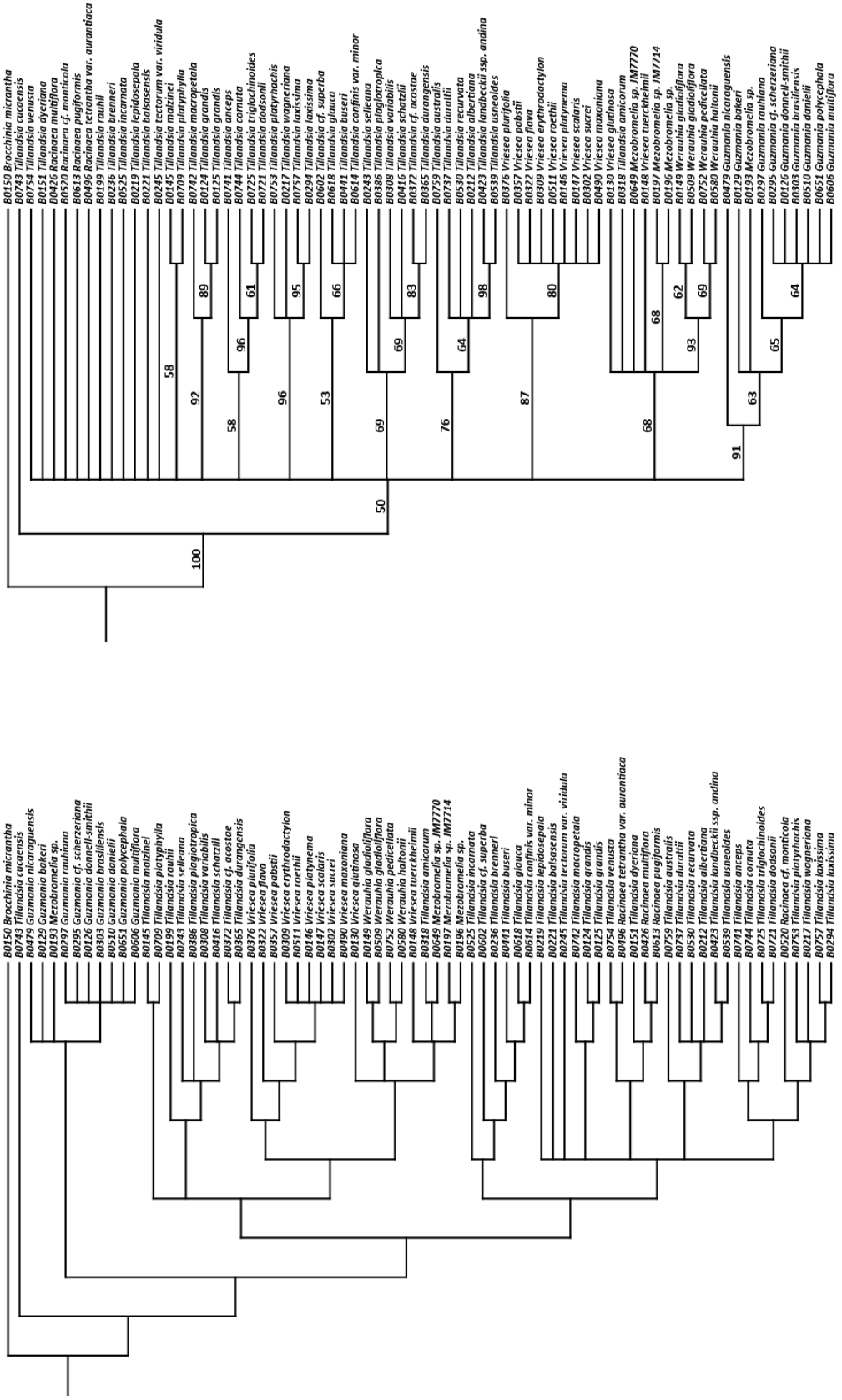


Figure 7. Strict consensus tree (A) and bootstrap consensus tree (B) of *NIA*.

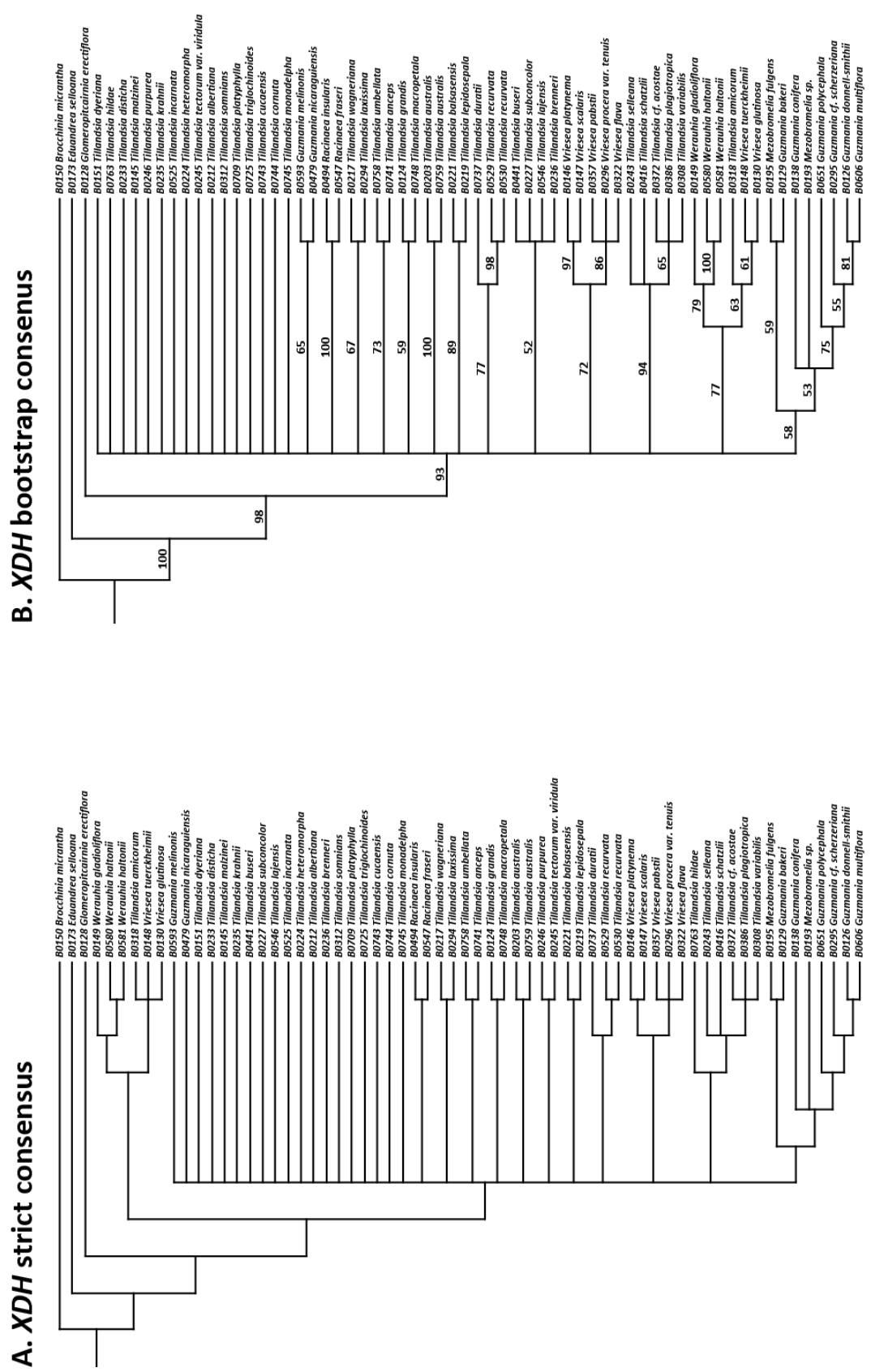


Figure 8. Strict consensus tree (A) and bootstrap consensus tree (B) of *XDH*.

NIA was investigated for an enlarged sample set (72 accessions). The amplified fragment (729–798 bp) covered a region from parts of exon 1 (629–638 bp), intron 1 (of variable length), to partial exon 2 (15 bp) (Figure 1, E). Fragment length ranged from 649–800 bp (*Brocchinia micrantha*, *Tillandsia duratii*, respectively). Some accessions displayed a microsatellite at the 3' end of intron1. A strict consensus and a bootstrap consensus tree are shown in Figure 7. Backbone relationships are not resolved, but several terminal clades receive support.

Xanthine dehydrogenase (XDH)

Amplification of *XDH* (Figure 1, F) was attempted using primers that were newly designed for partial exon 4 and complete intron 5 using sequences downloaded from GenBank. Primers of the phylogenetic studies of Górniak & al. (2010) and Morton (2011) became available only after the current study was completed. Amplification of intron 5 failed so only the successfully amplified part of exon 4 was further optimized (Table 2). Best amplicons were achieved using the primer combination “xdh479f”/”xdh1611r”. These were initially also used as sequencing primers, but in addition internal sequencing primers were developed to produce acceptable traces for the whole fragment. All primers are listed in Table 8.

XDH was investigated for an enlarged sample set (64 accessions). PCR primers produced fragments with part of exon 4 (1,107–1,110 bp). No indels within accessions were found, and only a 3-bp indel for *Brocchinia micrantha* was observed in the entire DNA matrix. Resolution is restricted to some terminal clades (Figure 8).

Phosphoribulokinase (PRK)

For *PRK* (Figure 1, G) initial gradient PCR runs were performed using degenerate primers originally designed for angiosperms, monocots, and/or palms (Lewis & Doyle 2002; D. Springate, RGB Kew, London, unpublished). Primer combinations “prk663f”/”prk1040r” and “prk663f”/”prk1167r” spanning a region of partial exon 2 to partial exon 5 amplified single, weak PCR products only in samples having high-quality DNA extracts, whereas any combination using “prk488f” located in exon 1 yielded no amplification. After successful sequencing of PCR products, a second set of bromeliad-specific PCR primers (“prk622f”, “prk1069r”) and additional internal primers (“prk734f”, “prk889r”) sitting in exon 4 were designed, which were initially used for Bromelioideae (Bromeliaceae) by Schulte & al. (2009). However, these primers often showed primer-dimer formation. Therefore in a last phase of optimization, three primers were slightly modified (“prk621f”, “prk890r”, “prk1069r-2”) to allow higher annealing temperatures and two additional nested sequencing primers (“prk630f”, “prk1057r”) were developed to avoid sequencing problems caused by incorporation of dimers into the products. For the earliest diverging genus of Bromeliaceae, *Brocchinia*, “prk734f” had to be adjusted because of mismatches present at the 3' end (prk734f-2). The optimized PCR conditions and all primers tested are given in Table 3 and Table 9, respectively.

Final PCR primers (“prk621f”, “prk1069r-2”) amplified fragments ranging from 831–1,692 bp in *Tillandsia ionochroma* and *Guzmania patula*, respectively (see Supplementary Data of chapter 5). A standard *PRK* sequence was composed of parts of exon 2 (9 bp), intron 2 (of variable length), exon 3 (85 bp), intron 3 (of variable length) exon 4 (245 bp), intron 4 (of variable length) and parts of exon 5 (108 bp) (Figure 1, G). Primers “prk621f” and “prk1069r-2” were designed to be highly specific for *PRK* of Bromeliaceae, yielding clean, single PCR bands even for accessions for which initial and second primer sets produced faint or no bands.

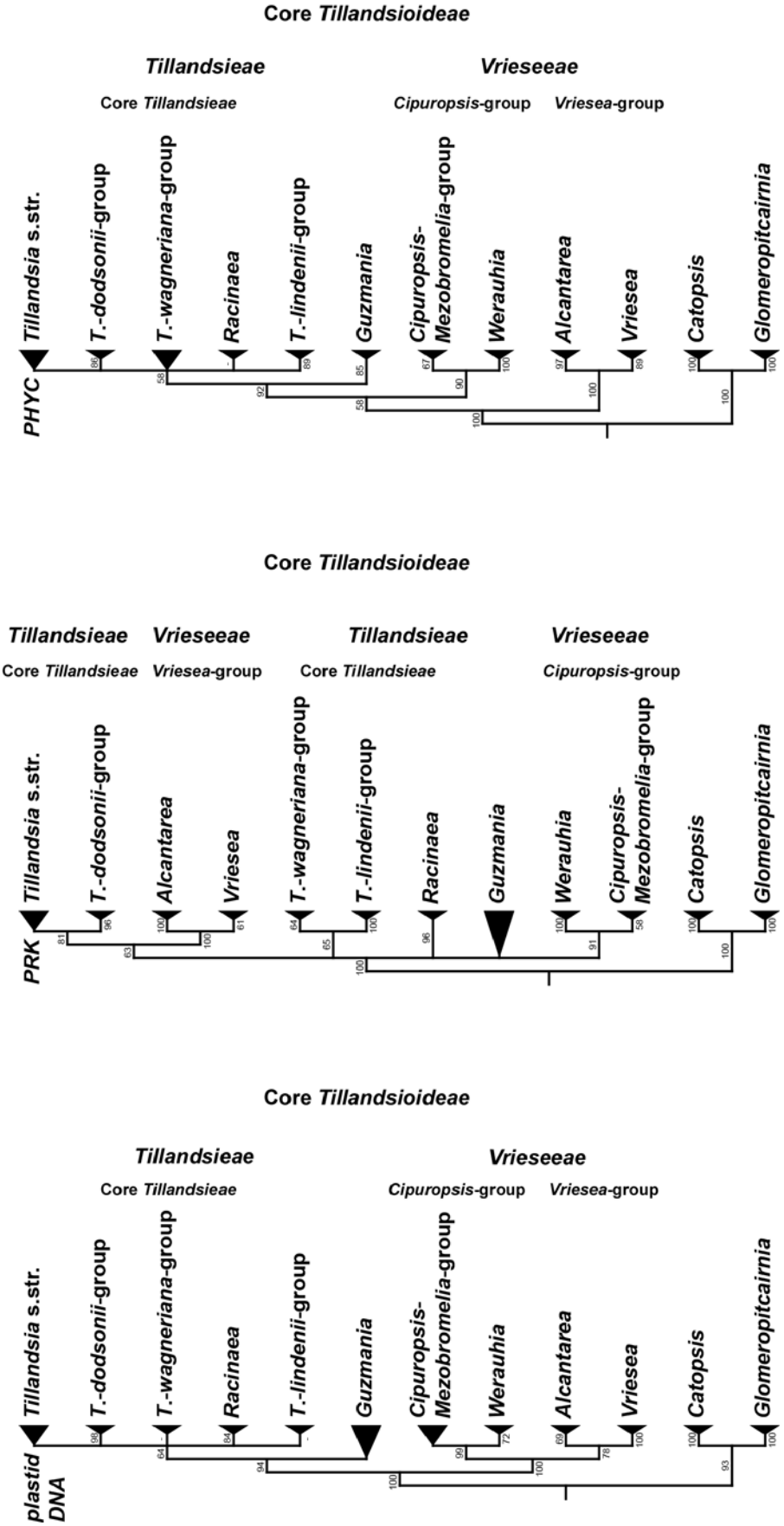


Figure 9. Simplified strict consensus trees of equally-most-parsimonious trees found in parsimony analyses of individual datasets supplemented with bootstrap support from parsimony bootstrap analysis either above or below branches; triangles sitting in terminal position on a branch indicate monophyletic groups, whereas triangles emerging from within a node indicate several branches of that group on a polytomy. The tree for plastid DNA was modified according to results of Barfuss & al., (2005) and is shown for comparison.

Direct sequencing was successful in about ⅔ (67.2%) of the accessions with few SNPs (23.6%). Intra-accession indels were detected in 146 (32.8%) accessions. The number of indels within a given accession ranged from 1–5. Indel variation was only found in the less conserved introns and never in exons. Three taxa had to be cloned, since the number of indels was too great to be unambiguously edited: *Vriesea psittacina*, *Guzmania graminifolia*, and *Tillandsia platyrhachis*. Occasionally others were cloned to unambiguously verify the length of both alleles [see Appendix]. SNPs were found in 241 (54.3%) out of 444 sampled accessions. Only 193 (43.5%) samples were found to be homozygous for the region sequenced; inter- and intra-individual variation in introns was high. The number of SNPs in a given accession ranged from 1–18 (highest number in *Tillandsia fasciculate*, B0076) were primarily found in introns and less frequently in exons, which primarily had synonymous substitutions. Homopolymers were found in several accessions in intron 2 and 4, respectively (see Supplementary Data, chapter 5).

Statistics for the *PRK* matrix and trees are given in Table 11. Indels are common at all hierarchical levels, yielding a final matrix of 3,291 characters. Evaluation of SNPs and indels within accessions showed no significant impact on subsequent data analysis (signal conflict), although homoplasy was present. Therefore coded SNPs were used except for the three cloned taxa.

Phylogenetic relationships based on *PRK* variation are shown in Figure 9, which is the strict consensus tree with bootstraps indicated. Strongly supported are the sister group relationship (BP 100) of *Glomeropitcairnia* (BP 100) and *Catopsis* (BP 100) and core Tillandsioideae (BP 100). Tribes Vrieseeae and Tillandsieae as well as core Tillandsieae are not supported. The *Cipuropsis* and *Vriesea* groups are strongly supported (BP 91 and 100, respectively). *Werauhia* is strongly supported (BP 100), whereas the *Cipuropsis-Mezobromelia* group has only weak BP (58). The same picture is seen for *Alcantarea* (BP 100) and *Vriesea* (BP 61). *Vriesea* is monophyletic only when Andean members located in the *Cipuropsis-Mezobromelia* group, *Tillandsia malzinei* and former xerophytic gray-leaved members, the latter two now placed within *Tillandsia* s. str., are excluded (data not shown). The sister group position of the *Vriesea* group and a clade containing *Tillandsia* s. str. and the *T. dodsonii* group was surprising but only weakly supported (BP 63). Monophyly of *Guzmania* is not supported, and three clades of *Guzmania* species (data not shown) occur in a polytomy with several others. The *T. wagneriana* (BP 64) and *T. lindenii* (BP 100) groups are resolved as sisters (BP 65). *Racinaea* is strongly supported (BP 96), but its relationships to other groups remain unresolved. *Tillandsia* s. str. and *T. dodsonii* group are resolved as sister groups with a moderate support (BP 81) with the *T. dodsonii* group being strongly supported (BP 96), but unresolved with several other clades of *Tillandsia* species.

Phytochrome C (PHYC)

For partial exon 1 of *PHYC* (Figure 1, H), initial PCRs were performed using the four degenerate primers “phyc0503f-mo”, “phyc0515f-mo”, “phyc1699r-mo”, and “phyc1705r-mo”, which were obtained from primer sequences originally published by Mathews & Donoghue (1999) and modified according to monocot sequences downloaded from GenBank. All primer combination worked, but showed strong primer-dimer formation. Best amplifications were achieved with “phyc0515f-mo” and “phyc1699r-mo”. However, all combinations were successfully sequenced, but showed strong signals of dimers at the start of the traces. Therefore bromeliad-specific PCR primers (“phyc0515f-br”, “phyc1699r-br”) were produced and optimized (Table 2) based on sequence information obtained from “phyc0503f-mo” and “phyc1705r-mo” since

their annealing position is outside the annealing positions of these primers. In addition, nested sequencing primers ("phyc0524f-br", "phyc1690r-br") were designed to avoid sequencing problems as much as possible. Several internal primers creating overlapping reads towards the end of the fragment were also developed to sequence the whole PCR product. Monocot-specific ones were also tested successfully for *Polystachya* (Orchidaceae) by Russell & al. (2010). Four internal primers giving the best reads were finally selected for sequencing. Details of all primers are given in Table 10.

Only fragments of partial exon 1 (Figure 1, H) were amplified ranging from 1,159–1,192 bp in *Werauhia insignis* and *Guzmania acorifolia*/*G. condensata*, respectively (see Supplementary Data of chapter 5), but mostly 1,177 bp. Direct sequencing was straightforward in nearly all cases. In contrast to *PRK*, no length differences between the two alleles of an accession were detected; this is likely due to the fact that only conserved parts of the exon 1 were amplified in *PHYC*. Only two obvious hybrids of distantly related taxonomic groups, where one of the possible parental taxa displayed two 3 bp insertions, were observed; these were finally excluded. Sequences of *PHYC* generally showed clean raw data with no or only few polymorphic sites in the electropherograms. Most individuals are either homozygous or show only little intraspecific allelic variation, as can be expected for relatively conserved exon. SNPs were found in 220 taxa out of 444 sampled individuals. About 224 samples were found to be homozygous for the amplified region. The number of SNPs in a given heterozygous taxon ranged from 1–15 (the upper limit seen in *T.* aff. *cucaensis* Wittm. B0735, see Supplementary Data of chapter 5). Distribution of polymorphic sites was even across the amplified portion of *PHYC* with no particular preference for a specific region, but significantly higher at the 3rd codon position (i.e. synonymous substitutions).

Information on the analyzed *PHYC* matrix and trees are given in Table 9. Indels are rare, and present either in single species or few closely related species, leading to a final matrix size of 1,228 characters. Like in *PRK*, all polymorphic sites have been checked for their impact on subsequent data analysis, which did not show significant conflicts. Therefore the polymorphism-coded *PHYC* dataset was used for all analyses, with the exception of three taxa, which were cloned for *PRK* and therefore also for *PHYC*, to allow the combination of corresponding allelic sequences.

Figure 9 shows the strict consensus tree of a MP analysis with BP, which summarizes phylogenetic relationships based on *PHYC* sequence data. As in *PRK* tree, both *Glomeropitcairnia* and *Catopsis* are strongly supported (BP 100) as sisters (BP 100), with the latter clade sister to a strongly supported core Tillandsioideae (BP 100). Tribe Vrieseeae is paraphyletic, but the resulting grade has only a weak support (BP 58). The *Vriesea* group (BP 100), *Alcantarea* (BP 97) and *Vriesea* (BP 89) are monophyletic, the last as found in *PRK*, but only when the Andean members, *Tillandsia malzinei* and former xerophytic gray-leaved species are excluded (data not shown). The Cipuropsis group splits into two lineages, *Werauhia* (BP 100) and the *Cipuropsis-Mezobromelia* group (BP 67). *Mezobromelia* is weakly supported (BP 57) as nested in the *Cipuropsis-Mezobromelia* group (data not shown), which in addition contains taxa previously assigned to *Vriesea* and *Tillandsia* (data not shown). Tribe Tillandsieae are strongly supported (BP 92). The first lineage that splits from core Tillandsieae is *Guzmania* (BP 85), with *Mezobromelia hutchisonii* nested inside (data not shown). Core Tillandsieae is only weakly supported (BP 58) and relationships of genera and groups are unresolved. The *T. lindenii* group (89 BP), *Racinaea* (no BP) and the *T. dodsonii* group (BP 86) are monophyletic but all three are placed in a polytomy with different clades of *Tillandsia* s. str. and the *T. wagneriana* group.

Discussion

Challenges of nuclear DNA markers

The fact that nuclear DNA sequences are much less used than plastid ones became evident in initial phases of the current study. Several problems highlighted earlier were also encountered during the optimization of nuclear markers for Tillandsioideae. The most time-consuming steps were primer design, extraction of high-quality DNA, PCR optimization, improving sequencing results of ITS nrDNA, and editing as well as aligning intron-containing markers and ITS nrDNA. The only advantage of Tillandsioideae is that it presumably consists of more than 98% diploid species, which clearly helps in data collection, evaluation and interpretation. The major challenges of nuclear DNA sequences for phylogenetic studies in Tillandsioideae are: (1) limited availability of effective primers; (2) occurrence of strong secondary structure (ITS nrDNA, *NIA*) that make PCR and sequencing difficult; (3) a short fragment length and consequentially a small number of PICs (e.g., amplified fragments of *MS*, *RPB2*); (4) heterozygous individuals with SNPs and indel mutations, the latter mainly in markers containing polymorphic introns (e.g., *PRK*), making sequence editing time-consuming and increased laboratory costs if cloning is required; and (5) alignment of markers containing polymorphic introns with indel variation. A proper pre-evaluation of nuclear DNA markers and the selection of more conserved regions (e.g., *PHYC*, *PGIC*, *XDH*) would have minimized these complications. A review article on nuclear DNA markers used in the Department of Systematic and Evolutionary Botany at the University of Vienna for different angiosperm lineages is in progress (Barfuss & al., in prep.).

Nuclear versus plastid DNA markers

The current results support the hypothesis that several nuclear DNA markers evolve much more rapidly than do plastid markers. The number of potential PICs per sequenced base pair in *PHYC* is 2-fold, in *PRK* 3-fold higher than in plastid DNA markers, which means sequencing these more variable nuclear DNA markers requires much lesser sequencing effort to have the same level of resolution as combined plastid DNA markers. However, homoplasy in three out of five nuclear data sets examined here was also higher (Table 11). Conserved regions like *XDH* evolve at about the same rate as plastid regions. Barfuss & al. (2005) and Givnish & al. (2011) have shown that the combination of up to eight plastid markers is insufficient to resolve phylogenetic relationships of Bromeliaceae down to the subgeneric rank (Figure 9). Inclusion of properly chosen plastid markers will definitely improve the already published plastid analyses, but this is costly and time-consuming. Whether sequencing more plastid or additional nuclear markers is more efficient and useful for the scientific questions being investigated needs to be evaluated before large datasets are sequenced.

Phylogenetic utility of nuclear markers

ITS nrDNA is not suitable for well resolved backbone relationships, although a lot of intra-individual variation was present (Figure 6). This is remarkable, since in many other angiosperm lineages (e.g., Barnadesioidae (Asteraceae): Gruenstaeudl & al., 2009) and especially in monocots (e.g., Musaceae: Hřibová, 2011; *Polystachya* (Orchidaceae): Russell & al., 2010;), ITS nrDNA has been applied successfully below family level. The strong secondary structure could have ecological significance for adaptation to hot and dry environments (Hurst & Merchant, 2001), as epi- and lithophytes are often exposed to changing microclimatic conditions. Howev-

er, orchid twig epiphytes in subtribe Oncidiinae that occupy the same micro-environments as many species of *Tillandsia* exhibit much higher levels of ITS nrDNA variation than do those species occupying more mesic epiphytic habitats (Chase, 2009). In this context, it is interesting to note that mesophytic bromeliad taxa (e.g., *Vriesea*, *Werauhia*) display a shorter problematic ITS1 region than xerophytic species (*Tillandsia*). Because of low resolution and great difficulties in amplification, sequencing and alignment, ITS nrDNA cannot be recommended as marker generally applied in phylogenetic studies of Bromeliaceae, but might be currently the only marker to discriminate species for barcoding purpose (Michael H. J. Barfuss, unpublished data).

Amplified fragments of *MS* and *RPB2* are relatively short and information content is limited compared to other markers studied. Therefore these genomic regions were not further investigated for an enlarged set of samples. Although *PGIC* seems promising regarding ease of sequencing and alignment at various taxonomic levels within Bromeliaceae, it was not further tested for other samples of Tillandsioideae. Trees of *NIA* and *XDH* show limited resolution of deeper nodes (Figures 7, 8, respectively). However, several terminal clades receive bootstrap support, and both markers might be useful in adding more phylogenetic information in a combined analysis of nuclear DNA markers. Results of *XDH* show that this marker is better suited to higher taxonomic levels (as in Orchidaceae; Gorniák et al., 2010), whereas *NIA* might help better discriminate closely related taxa.

Preliminary results indicated that *PRK* and *PHYC* were the two most promising nuclear DNA markers tested in terms of amplification and sequencing ease as well as in providing high numbers of PICs. Therefore these regions were sequenced for all available Tillandsioideae accessions. As for combined plastid data, individual nuclear DNA markers are unable to resolve relationships completely (Figure 9). Although several clades are retrieved and highly supported by most markers, they still provides different degrees of resolution and levels of support below the subfamily rank. *PHYC* is easy to align across the analyzed taxa, but it fails to provide resolution in more terminal nodes, especially in core Tillandsieae. *PRK* is difficult and not suitable, especially at higher levels, in the light of the numerous indels that have to be introduced into the alignment, but it helps to provide more resolution at intermediate levels.

Recently diverged species

To address questions concerning relationships of recently diverged species within genera of Tillandsioideae, our sampling was far too limited and turned out to be problematic in taxonomically complicated species complexes, especially when consensus allelic sequences are used. However, in the complete data sets of *PRK* and *PHYC* we sometimes included two or sometimes more accessions of the same taxon, which mostly cluster together or into clades of closely related species. We also found some cases where accessions of the same species but of a different subspecific rank did not cluster, although these accessions were still not far from each other. Whether these patterns are results of missing PICs, homoplasy, allelic consensus sequences, or questionable species concepts would need to be investigated further. It is clearly necessary to include multiple accessions of the same species/taxon, if possible over their whole distribution, and preferably complete gene sequences including all exons and introns to gain more linked information. In addition more cloning for divergent alleles of an individual, especially in complicated species complexes, is required to have pure allelic data.

Hybrids, hybrid speciation and reticulate evolution

The two species suspected to be of hybrid origin, *T. dorotheae* and *T. marconae*, could be clearly confirmed by sequencing nuclear DNA. Detection was possible since parental taxa belong to different lineages within *Tillandsia* with relatively high sequence divergence. The two alleles of each sample cluster with high support with the clade of their parents. Parents of *T. dorotheae* are *T. albertiana* and a species of the *T. argentina* complex, that of *T. marconae*, *T. landbeckii* and a species of the *T. purpurea* complex. Most living plants in the botanical gardens or private collections are of vegetatively propagated origin from one or a few wild collections. Whether these species are allopolyploids, homoploid hybrids, or just F_1 individuals of an occasional recent hybridization event would need to be investigated by more documented material. Except for these two cases, we currently do not have well-supported instances of hybridization, reticulate evolution or incomplete lineage sorting in other species.

Acknowledgements

The authors thank Tod F. Stuessy for providing lab facilities; many botanical gardens and individual people for providing plant material; Walter Till for taxonomic expertise, Mark W. Chase for a critically review of the manuscript. Financial support was provided by the University of Vienna, the Commission for Interdisciplinary Ecological Studies (KIÖS) at the Austrian Academy of Sciences (ÖAW) to W. Till and M.H.J. Barfuss (2007-02), and the SYNTHESYS (Synthesis of Systematic Resources) program funded by European Union.

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Part 3

Classification of Bromeliaceae subfamily Tillandsioideae

Chapter 4

Progress towards a new classification of Tillandsioideae

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Status: published, *Journal of the Bromeliad Society* 56 (6): 253–259. 2006.

Contribution: data collection, data analysis, manuscript writing/editing

Scientific

Progress towards a new classification of Tillandsioideae

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Introduction

The latest available monograph of Bromeliaceae (Smith and Downs 1974, 1977, 1979) classifies the family into three subfamilies among which Tillandsioideae comprises six genera. These are defined by the position of the ovary (superior vs. semi-inferior in *Glomeropitcairnia*) and seed appendage morphology (straight and basally elaborated vs. folded and apical in *Catopsis*) while the remaining four genera are characterised mainly by corolla morphology: free petals with basal appendages in *Vriesea*, the same but without appendages in *Tillandsia*, fused petals with appendages in *Mezobromelia* and the same but unappendaged in *Guzmania*. A few exceptions from these definitions have been found in *Vriesea* species that are without appendages or with basally fused petals. Thus the generic definitions provided by the last monographer were soon questioned. It has been demonstrated that especially petal appendages are developed late in ontogeny (Brown and Terry 1992) and that perhaps single mutations are sufficient to suppress their development. Consequently such characters are of little value to define genera.

More than 15 years ago molecular data first challenged Smith and Down's notion...

In the past two decades molecular phylogenetic studies have revolutionised the classifications of living organisms and have demonstrated that numerous characters hitherto used by taxonomists have either evolved several times in a parallel way or have independently been lost. In both ways relationships have been suggested without a natural basis, and those classifications are now recognised to be artificial.

Bromeliaceae are no exception. More than 15 years ago molecular data first challenged Smith and Down's notion that Bromeliaceae consists of just three subfamilies (Ranker et al. 1990) and *Brocchinia* was soon recognised as an early diverging lineage (Terry et al. 1997a) being sister taxon to the rest of the family. In Tillandsioideae, *Catopsis* and *Glomeropitcairnia* were identified as sister taxa to the rest of the subfamily which was called the „core" group (Terry et al. 1997b). It is this latter assemblage of genera which is still not sufficiently understood.

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Material and methods

To resolve relationships in Tillandsioideae and to define natural genera we have conducted a comprehensive molecular study at the Institute of Botany of the University of Vienna which resulted in more than 100 studied tillandsioid species (Barfuss et al. 2005). Attention was paid to include as many generic type species as possible and to select representatives from all morpholgically divergent groups. In this attempt we succeeded in most but not all cases. We focussed on the chloroplast genome which is not responsible for morphological characters and is therefore free of these functional constraints, and selected cpDNA regions with different rates of evolution (some conservative vs. more rapidly evolving ones). Technical details are found in Barfuss et al. (2005).

Results

Our results (Barfuss et al. 2005) confirm the sister taxon position of *Catopsis* and *Glomeropitcairnia* to the rest of the subfamily but they also demonstrate that none of the genera of Smith and Downs (1977) assigned to the „core group" by Terry et al. (1997b)

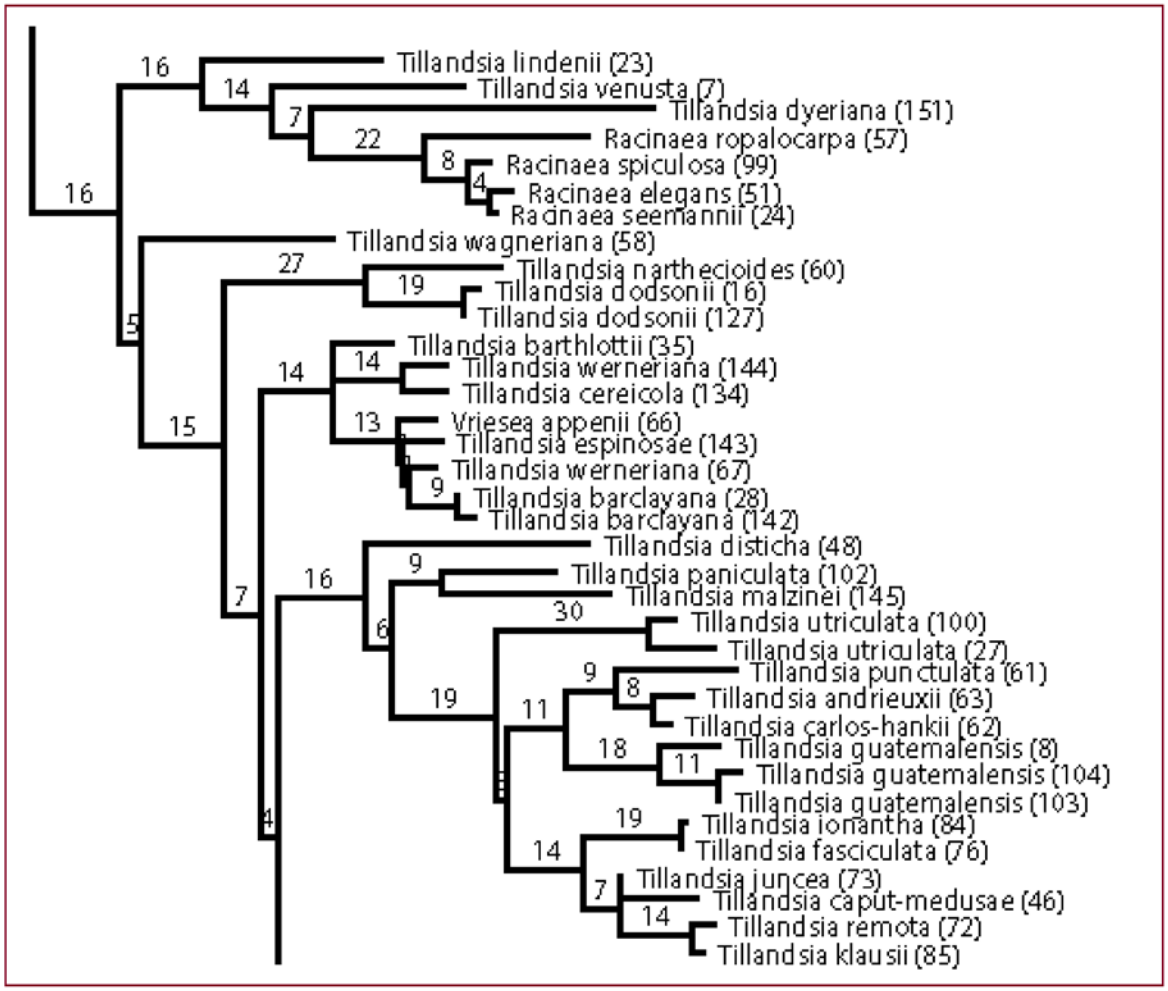

Figure 1. Section of Phylogram containing *Tillandsia* species.

is monophyletic, i.e., represents a natural group, in the circumscriptions provided by the monographer. *Vriesea* in our analysis is split into an East Brazilian and an Andean clade, but the two sections of Smith and Downs (1977) are not supported. *Alcantarea* is monophyletic and Grant's (1995) resurrection of this genus is justified. *Werauhia* is monophyletic with *Vriesea splendens* and *V. monstrum* in basal position. However, using an enlarged taxa set (Barfuss, unpubl.) these two species are removed from *Werauhia* and corroborate the monophyly of the latter. This is a good example of how insufficient sampling can influence the results. *Guzmania* appears to be monophyletic with *Sodiroa* well nested within in a terminal position. However, *G. bakeri* together with *Mezobromelia hutchisonii* froms a sister clade to *Guzmania* plus *Tillandsia* s. l. Again, sampling is insufficient in both genera involved and the pattern may change, but *Sodiroa* is not supported as a separate genus.

Tillandsia is the most complex genus and none of its subgenera is supported by the

Figure 2. Continuation from Figure 1 of section of Phylogram containing *Tillandsia* species.

molecular data. Relative length of stamens and styles to the corolla, petal shapes and filament plications—characters which have been the basis for defining the subgenera of Smith and Downs (1977)—all seem to be adaptations to pollinators rather than reliable taxonomic characters. Figs. 1 and 2 (continuation of fig. 1) show the fraction concerning *Tillandsia* from a phylogram of one of several most parsimonious trees generated from 136 accessions. Albeit in general the same branching pattern as in Barfuss et al. (2005) is visible some exceptions are remarkable. For example, *T. viridiflora* is no longer sister taxon to the rest of the genus but is nested within it in a central position together with *T. grandis*. However, the remaining two species of *subgen. Pseudalcantarea* are found in completely different branches: *T. paniculata* at the basis of a clade representing nearly exclusively members of *subgen. Tillandsia* while *T. baliophylla* comes close to *T. fendleri* within a green-leaved *subgen. Allardtia* clade. These results are in conflict with the classification of *subgen. Pseudalcantarea* proposed by Beaman and Judd (1996).

Within *Tillandsia*, sensu lato mesic members of *subgen. Phytarrhiza* including *Racinaea* constitute the earliest diverging clades, far remote from the xeric members. *T. wagneriana* stands as a separate clade and this position is supported by the „wagneriana seed type" (Groß 1988). Xeric members of *Vriesea*, which have recently been classified under *Tillandsia*, form a clade of their own near *subgen. Tillandsia* which is the most homogeneous clade with the exception of *T. rauhii* and *T. multicaulis* which are nested within species of *subgen. Allardtia*. *T. marconae* and *T. cacticola* as well as *T. tectorum*, *Viridantha plumosa* and *V. tortilis* form separate clades branching off before a terminal bulk which contains the majority of *subgen. Allardtia*, all members of *subgen. Anoplophytum*, xeric species of *subgen. Phytarrhiza*, and *subgen. Diaphoranthema*. It is evident from figs. 1 and 2 that none of the subgenera is monophyletic, and new groupings have to be defined in order to name natural taxa. *Viridantha* (Espejo-Serna 2002) appears to be monophyletic with the Andean *T. tectorum* in sister taxon position but more taxa need to be studied.

We have started to map onto our phylogenetic tree characters of which we are fairly convinced that they are not, or only to a moderate degree, subjected to adaptive constraints: pollen, stigma, and ovule morphology. Our results exhibit some very interesting trends and make us optimistic that combinations of new or under-utilised morphological characters can define natural groups as they are increasingly emerging from the molecular data.

Catopsis and *Alcantarea* are well supported by the „catopsis-„ and „alcantarea-pollen type" respectively. Both have massive and more or less clear cut aperture margins but no exine fragments on the aperture itself. By contrast, the most common pollen is of the „diffuse type" where the exine is irregularly dissolved at the aperture margins and fragments of the reticulum are spread over the aperture. This kind of pollen is typical for *Glomeropitcairnia*, *Mezobromelia*, and most *Guzmania* (a few species have inaperturate pollen), in Andean *Vriesea*, in former xeric *Vriesea* and in *subgen. Tillandsia*. It is less frequent in *subgen. Allardtia*. Similarly common is the „insulae-type" which is typical

for East Brazilian *Vriesea*, *Werauhia*, *T. appenii*, and many members of *subgen. Allardtia* (including the *Viridantha-T. tectorum* clade) and *Anoplophytum*. In the latter subgenus plus in *T. alberiana* and *T. xiphioides* the „operculum type" occurs. *T. viridiflora* and *T. grandis* have their own „Pseudalcantarea-type" in which the aperture is covered by a flat and continuous reticulum thus approximating to the inaperturate pollen. Morphological progressions appear to go from firm aperture margins and naked apertures toward dissolution of the aperture margins and to covered apertures via fragments of reticulum and exine insulae which finally form a compact operculum (like a shield), or to aperture closure (inaperturate). The different pollen types are not randomly distributed but are highly group specific in the lower parts of the phylogram and less so in the terminal parts.

Stigma morphology is another promising set of morphological characters, for terminology see Brown and Gilmartin 1984, 1989. *Catopsis* is characterised by simple-erect stigma branches which may somewhat twist and then resemble the conduplicate-spiral type. *Glomeropitcairnia* has convolute-blade stigmas like East Brazilian *Vriesea* and *Alcantarea* but with less pronounced papillae. By contrast, Andean *Vriesea* has simple-erect or conduplicate-spiral stigmas, those of *Werauhia* are cupulate (a cup shaped simple-erect stigma lacking papillae). *Mezobromelia* and *Guzmania* are very similar with simple-erect stigmas, only few species of the latter have convolute-blade stigmas. In *Tillandsia* s. l. the coralliform stigma stype appears to be unique for mesic members of *subgen. Phytarrhiza* (but see also under xeric members!). As far as studied former grey *Vriesea* and *subgen. Tillandsia* uniformly exhibit conduplicate-spiral stigmas (*T. malzinei* excepted) with simple-erect ones. Stigmas of *T. viridiflora* and of *T. grandis* are conduplicate-spiral. In subgenera *Allardtia*, *Anoplophytum*, and *Diaphoranthema* and in xeric members of *sugben. Phytarrhiza* the simple-erect stigmas prevail. However, it should be stressed that this stigma type is likely to be a heterogeneous assemblage including simplifications of the convolute-blade, the conduplicate-spiral, and of the coralliform types. Although in *T. latifolia* the stigma lobes have the shape of a shoehorn and are simple-erect in the original sense (Brown and Gilmartin 1984), stigma lobes of xeric members of *subgen. Phytarriza* and of *subgen. Diaphoranthema* have lateral auricles suggesting that these stigmas actually are strongly simplified coralliform ones. It is also often hard to decide whether a stigma is still simple-erect or already conduplicate-spiral or the reverse. However, the trends are clear and in combination with other characters stigma morphology is likely to be of help in defining genera.

Finally we compared ovule morphology with our molecular data. *Glomeropitcairnia* has ovules with extremely long slender appendages on both ends which is a unique situation in the subfamily. Similarly outstanding are the ovules of *Catopsis* which are usually chlorophyllous and bear a longitudinally divided apical coma which develops into multicellular folded hairs in fruit. In the „core group" ovule appendages, if present, are always apical and undivided. In *Alcantarea* these are distinctly longer than the ovule itself. In East Brazilian *Vriesea* appendages are as long as the ovule or somewhat shorter,

and in Andean *Vriesea* the appendages are lacking or are minute as in some *Werauhia* species. *Guzmania* and *Mezobromelia* are uniform in the absence of apical appendages and their ovules are usually slenderly cylindric. Ovules of mesic members of subgen. *Phytarrhiza* and of *Racinaea* are strikingly similar to those of *Guzmania* and *Mezobromelia*. In *subgen. Tillandsia* appendages are as long as the ovule itself *T. malzinei* excepted which is lacking the appendage. Xeric former-*Vriesea* have appendages somewhat shorter than the ovule itself and contrast with *subgen. Tillandsia*. *T. viridiflora* has a minute appendage while that of *T. grandis* is about as long as the ovule. *T. marconae* and *T. cacticola* have short appendages like the xeric members of *subgen. Phytarrhiza* and *subgen. Diaphoranthema*, all being different from the unappendaged ones in mesic members of *subgen. Phytarrhiza*. In subgenera Allardtia and Anoplophytum distinctly to short appendaged ovules are the rule but some species lack appendages. Like in the previous characters, some clades, especially those in the lower part of our phylogenetic tree, are well corroborated by ovule morphology but significance decreases in the upper portion of the phylogram.

Proposed genera *Racinaea* and *Viridantha* are not supported...

These preliminary data demonstrate that there are morphological characters suited to define natural entities at the generic level. We need more taxa to be studied to strengthen the phylogenetic backbone and we need to complete the morphological datasets. There are more promising characters like seed morphology (Groß 1988), stamen morphology and filament insertion at the anther, petal appendage morphology, stigma papillae etc. However, some of these characters should be re-evaluated before being used for taxonomy

Conclusions

Several published molecular phylogenies of subfam. Tillandsioideae clearly demonstrate that the classification in the latest monograph is artificial. Traditional morphological characters fail to define the clades emerging from the DNA based studies. While *Catopsis* and *Glomeropitcairnia* as genera are out of discussion and *Alcantarea* and *Werauhia* increasingly are corroborated, *Vriesea* is clearly separated into an East Brazilian and an Andean clade, the boundaries between *Mezobromelia* and *Guzmania* fade, and *Tillandsia* s. l. remains to be the most critical group. Proposed genera *Racinaea* and *Viridantha* are not supported in their current circumscriptions, and former xeric *Vriesea* fall into *Tillandsia* s. l. but constitute a separate clade. From the six subgenera currently classified under *Tillandsia* s. l. only *subgen. Tillandsia* forms a rather homogeneous clade, all others are split. *Subgen. Allardtia* is located in the upper portion of our phylogram and includes *subgen. Anoplophytum*, the xeric members of *subgen. Phytarrhiza*, and *subgen. Diaphoranthema*, its type species *T. guatemalensis*, however, is firmly nested within subgen. *Tillandsia*. This makes *Allardtia* an obligate synonym of *Tillandsia*. *Subgen. Phytarrhiza* is split into five clades in our analysis and best indicates the dramatic rearrangement

needed in *Tillandsia* s. l. However, no nomenclatoric changes are recommended before a significantly higher number of taxa is studied and a more balanced sampling of the „core group" has been made, including all generic type species..

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Chapter 5

A new classification of Bromeliaceae subfamily Tillandsioideae inferred from DNA sequences data of two genomes and morphology

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Keywords: *Alcantarea*; *Catopsis*; *Cipuropsis*; plastid DNA; *Glomeropitcairnia*; *Guzmania*; *Josemania*; *Lectotypifications*; *Lemeltonia*; *Mezobromelia*; *PHYC*; *PRK*; *Racinaea*; *R*. subg. *Pseudophytarrhiza*; *Rothowia*; low-copy nuclear DNA; *Tillandsia*; *T*. subg. *Pseudovriesea*; *T*. subg. *Viridantha*; *Vriesea*, *Werauhia*

Running Title: New classification of Tillandsioideae (Bromeliaceae)

Status: in preparation, intended to be submitted to *Taxon*

Contribution: data collection, data analyses, manuscript writing

Abstract

In order to establish a natural and stable classification system for Tillandsioideae we conducted phylogenetic analyses of newly generated low-copy nuclear DNA sequence data (*PHYC*, *PRK*) and previously published plastid DNA markers (*atpB-rbcL*, *matK*, *rbcL*, partial *rbcL-accD*, *rps16* intron, partial *trnK* intron, *trnL* intron, *trnL-trnF*), together with a re-evaluation of morphological characters. Parsimony analysis revealed the following, mostly well-supported general branching pattern for Tillandsioideae: ((*Glomeropitcairnia*, *Catopsis*) (((*Alcantarea*, *Vriesea*) (*Werauhia*, *Cipuropsis-Mezobromelia* clade)) (*Guzmania*, ((*Josemania*, *Racinaea*) (*Rothowia*, (*Lemeltonia*, *Tillandsia*)))))). Core Tillandsioideae are well supported by all analyses, but Bayesian analysis yielded a slightly different branching pattern for core Tillandsieae: (*Guzmania*, ((*Josemania*, *Rothowia*) (*Racinaea* (*Lemeltonia*, *Tillandsia*))). Based on the results a new classification of Tillansioideae is presented. Two new subtribes (Cipuropsidinae, Vrieseinae), three new genera (*Josemania*, *Lemeltonia*, *Rothowia*), and three new subgenera (*Racinaea* subg. *Pseudophytarrhiza*, *Tillandsia* subg. *Viridantha*, *T.* subg. *Pseudovriesea*) are described and several species are reclassified. A key to the genera of Tillandsioideae is also provided. Lectotypes are selected for *Catopsis* subg. *Tridynandra*, *Tillandsia* sect. *Conostachys*, and *Tillandsia* sect. *Eriophyllum*. Classification of some unresolved phylogenetic units remains informal and needs further attention, especially within the *Cipuropsis-Mezobromelia* clade and the genus *Tillandsia.*

Introduction

Tillandsioideae Burnett is the largest and morphologically most diverse subfamily of Bromeliaceae Juss. with more than 1300 species in nine generally accepted genera (Smith & Downs, 1977; Smith & Till, 1998; Till, 2000a; Grant & Zijlstra, 1998; Luther & Rabinowitz, 2010; species numbers according to Luther, 2010): *Alcantarea* (E. Morren ex Mez) Harms (28 spp.), *Catopsis* Griseb. (18 spp.), *Glomeropitcairnia* (Mez) Mez (2 spp.), *Guzmania* Ruiz & Pav. (210 spp.), *Mezobromelia* L.B. Sm. (9 spp.), *Racinaea* M.A. Spencer & L.B. Sm. (65 spp.), *Tillandsia* L. (626 spp., in 6 subgenera), *Vriesea* Lindl. (266 spp., in two sections), and *Werauhia* J.R. Grant (87 spp., in 2 sections). The genus *Viridantha* Espejo (8 spp.; Espejo-Serna, 2002; Espejo-Serna & al., 2007; López-Ferrari & Espejo-Serna, 2009) is included in *Tillandsia*, following most recent authors, and is subdivided into two sections, sect. *Viridantha* (5 spp.) and sect. *Caulescens* Espejo (3 spp.).

A well-established taxonomic concept of the whole subfamily is of much interest, because tillandsioids are an important component of neotropical ecosystems accounting for a high portion of recorded (epiphytic) biodiversity, are of special importance for conservationists since many species are endemic to certain regions, have the greatest number of horticulturally important Bromeliaceae species, and are popular amongst bromeliad collectors and amateur botanists. Despite its importance, a well-supported and broadly accepted classification scheme has not been achieved to date. The main reasons are the lack of definite morphological characters to delimit genera, subgenera and sections, and the low variability of DNA markers studied up to now, weakening the possibility to reconstruct a solid phylogenetic framework with well-supported, monophyletic entities (see Hörandl & Stuessy (2010) for the definitions of evolutionary terms used in this study).

Traditional morphological classifications

A large number of species in the subfamily exhibit considerable interspecific morphological variation especially in reproductive organs (e.g., inflorescences and flowers) and in general habit, with life forms ranging from entirely mesophytic and water-impounding to strongly xerophytic, rarely terrestrial to commonly epiphytic or lithophytic (e.g., Gilmartin, 1983; Gilmartin & Brown, 1986; Benzing, 2000; Stefano & al., 2008). Early bromeliad taxonomists considered floral morphological characters to be most important for classification, since there is sometimes no diagnostic variation in the vegetative structures of distantly related species (Baker, 1889; Mez, 1896, 1934–35; Smith, 1951; Smith & Downs, 1977). Smith & Downs, authors of the last monograph of Bromeliaceae (Pitcairnioideae Harms: 1974; Tillandsioideae: 1977; Bromelioideae: 1979) maintained the use of these floral characteristics in their taxonomic treatments: the four main characters used to differentiate genera of Tillandsioideae are the position of the ovary, the structure of seeds, the presence vs. absence of petal appendages, and free vs. conglutinate petals. However, in their "Preface" Smith & Downs (1977: 663) state about the difficulty of identifying Tillandsioideae genera "once the crucial petals and stamens are lost" and the sometimes inconsistent placement of species by Mez (1934–35; cf. the different treatment of several xerophytic, grey-leaved *Vriesea* species by both authors). Therefore, many species are misplaced in both monographs according to these generic definitions, since their classification was often based on herbarium specimens without perfect flowers (e.g., Utley, 1978; Weber & Smith, 1983; Utley & Luther, 1991; Grant, 1993a).

Revisions of floral morphological characters used and neglected in Smith & Down's monographs pointed out the potential and limits of these features to classify Bromeliaceae species (petal appendages: Brown & Terry, 1992; stigmas and papillae: Brown & Gilmartin, 1984, 1989b; Schill & al., 1988; Gortan, 1991; septal nectaries: Böhme, 1988; pollen: Halbritter, 1988, 1992; and seeds: Gross, 1988). No taxonomic change was proposed in any of these studies, because species sampling was too limited to generally apply recognized characteristics to higher taxonomic units. Resurrections and segregations of subgenera and closely related species complexes at generic status were done later based on the consideration of these additional and reevaluated morphological characters (*Alcantarea*: Grant, 1995a; *Racinaea*: Spencer & Smith, 1993; *Werauhia*: Grant, 1995a; and *Viridantha*: Espejo-Serna, 2002). Some of these new genera are not always accepted, because remaining species of *Tillandsia* and *Vriesea* are left in their old generic circumscriptions without alteration of their morphological definitions. Even more surprising and hardly acceptable for some bromeliad specialists are rearrangements of former xerophytic, grey-leaved *Vriesea* species done by Grant (1993b, 1994b, 1995b, 2005), since the new placement clearly contradicts the definition of the genus *Tillandsia* established by earlier botanists, although for example Mez (1934–35) did not always follow strictly this characterization when new morphological evidence arose.

The controversies about the correct generic placement of certain species have led to the situation that modern bromeliad taxonomists follow different opinions regarding a generally accepted classification scheme (Grant & Zijlstra, 1998; Smith & Till, 1998; Till, 2000a, b; Barfuss & al., 2005; Luther & Sieff, 1994, 1997; Luther, 2001, 2008, 2010; Luther & Rabinowitz, 2010), since some of these modifications were based on morphological dissimilarity and not on a shared character or character combinations (see Tables 4 and 5 for a comparison of traditional and most recent classification systems). The most important morphological character at the generic level within Tillandsioideae (and Bromeliaceae in general) is the presence vs. absence of petal appendages (Smith & Downs, 1974, 1977, 1979; Brown & Terry, 1992), which delimits the genus pairs *Tillandsia*/*Vriesea* and *Guzmania*/*Mezobromelia*. The taxonomic value of this character has been questioned (e.g., Read, 1968; Utley, 1978; Gardner, 1982; Gilmartin, 1983; Beaman, 1989) although all bromeliad monographers used it in their taxonomic treatments (Wittmack, 1888; Baker, 1889; Mez, 1896; Harms, 1930; Mez, 1934–35; Smith & Downs, 1977). Field botanists discovered that within populations of a single species, individuals with and without petal appendages can coexist (Read, 1968; W. Till, pers. com.), which would mean, following the strict generic concept of Smith and Downs (1977), these individuals should be classified in different genera. Despite these problems with using petal appendages as a strict and definite character, Benzing & al. (2000) countered that "...the frequently disparaged petal appendage would regain some lost currency as one of the more useful among the traditional characters... [if] occasional exceptions" are accepted.

Defining infrageneric units morphologically (e.g., within *Tillandsia*: Till, 2000b) is an analogous difficulty. Only a few classical taxonomic studies have treated whole subgenera or species complexes of *Tillandsia*: *T.* subg. *Anoplophytum* (Tardivo, 2002), *T.* subg. *Diaphoranthema* (Till, 1984, 1992), *T.* subg. *Phytarrhiza* (Gilmartin, 1983; Gilmartin & Brown, 1986), *T.* subg. *Pseudalcantarea* (Beaman, 1989; Beaman & Judd, 1996), *T.* subg. *Tillandsia* (Gardner, 1982, 1986a, b; including some taxa from *T.* subg. *Allardtia* sensu Smith & Downs, 1977), the *Tillandsia gardneri* complex (Ehlers, 1997), *Tillandsia macdougallii* complex (Granados Mendoza, 2008), the *Tillandsia plumosa* complex (= *Viridantha*) (Espejo-Serna, 2002; Ehlers, 2009), and the *Tillandsia tectorum* complex (Hromadnik, 2005). *Tillandsia* subg. *Allardtia* was lastly only treated

within the "*Tillandsia* and *Racinaea*" chapter by Till (2000b). The separation of *T.* subg. *Allardtia* and *T.* subg. *Anoplophytum* is based on the character "straight vs. plicate filaments" and *T.* subg. *Tillandsia* is distinguished from both by its "exserted stamens and style". Till (2000b) stated that "the distinction between subgenera *Allardtia* and *Anoplophytum* is weak, and their separation may not be justified, at least not as proposed by Smith & Downs" (1977). The existence of closely related species (Till, 2000b) showing either of these subgeneric characteristics (straight vs. plicate filaments, respectively) and previous ontogenetic studies on filament plication (Evans & Brown, 1989) demonstrate that this distinction is inappropriate. This is reflected by the opinion of Till (2000b), where he moved the Andean species with plicate filaments from *T.* subg. *Anoplophytum* sensu Smith & Downs (1977) into *T.* subg. *Allardtia*. Several species of other subgenera of *Tillandsia*, especially of *T.* subg. *Pseudalcantarea* and *T.* subg. *Phytarrhiza* sensu Smith & Downs (1977), show similar patterns of morphological convergence or parallelism.

Since traditional generic, subgeneric and sectional treatments are exclusively based on flower morphology, the most plausible explanation for their evolution is that pollination syndromes can adapt relatively fast to new environmental situations, leading to floral characteristics which could have evolved in parallel or convergently in different phylogenetic lineages. This might be caused by a high capability of bromeliads in general to adapt easily to different ecological niches and modified environmental conditions (e.g., Gardner, 1986a; Benzing, 2000; Kessler, 2002; Schmidt-Lebuhn & al., 2007; Krömer & al., 2008). Despite known problematic issues of previously-used floral morphological characters, their usage still persists, since no other diagnostic characters or character combinations have yet been identified or are sufficiently known to unambiguously circumscribe natural taxonomic units.

Previous molecular phylogenetic studies

Molecular phylogenetic studies which included members of Tillandsioideae revealed a holophyletic (= monophyletic s.str.; see Hörandl & Stuessy, 2010) subfam. Tillandsioideae (e.g., Terry & Brown, 1996, Terry & al., 1997a, b; Horres & al. 2000; Crayn & al., 2004; Givnish & al., 2004; Givnish & al., 2007; Givnish & al., 2011). Also, the early diverging clades comprising *Catopsis* and *Glomeropitcairnia* were well supported in most studies. However, resolution in core Tillandsioideae genera (*Alcantarea*, *Guzmania*, *Mezobromelia*, *Racinaea*, *Tillandsia*, *Viridantha*, *Vriesea*, and *Werauhia*; = Tillandsioideae s.str., Terry & al., 1997b) was greatly lacking, because phylogenetic studies only relied on a single or few plastid DNA regions. First reliable insights into relationships of core Tillandsioideae were brought by the seven marker analyses of Barfuss & al. (2005). The main results of this study were the split of core Tillandsioideae into two main lineages and the para- and/or polyphyly of the largest tillandsioid genera *Tillandsia* and *Vriesea* based on the information content of these plastid DNA markers. All other lastly segregated genera were found to be holophyletic, but they were clearly nested either within *Vriesea* (*Alcantarea*, *Werauhia*) or within *Tillandsia* (*Racinaea*, *Viridantha*) according to their traditional generic circumscriptions. Taxonomic changes proposed since the monograph of Smith & Downs's (1977) were only partly supported and clearly showed, that morphology based taxonomy is in conflict with molecular results. However, the taxon sampling was too limited for making a conclusive decision regarding holophyly/paraphyly of these genera. The study also showed that none of the previously applied infrageneric concepts of *Tillandsia*, *Vriesea*, and *Werauhia* using traditional morphological characters were fully supported by plastid DNA sequence data indicating convergent or parallel evolution of certain features. A

preliminary report of Till & Barfuss (2006) including results of one single-copy nuclear gene (Phosphoribulokinase = *PRK*; not explicitly stated in the text but used to create Figures 1 and 2 of Till & Barfuss, 2006; 254–255) further corroborates these findings. However, the nested position within *Tillandsia* of meso- and semi-mesophytic taxa of *T*. subg. *Phytarrhiza* and *Racinaea* is no longer supported whereas *Viridantha* still remains nested within *Tillandsia*. Nevertheless, the shortcomings of all previous DNA studies were that they suffered from an extremely low number of parsimony-informative nucleotide characters (PICs), a serious undersampling of taxa in relation to the actually accepted species and the dependence on few analyzed plastid DNA regions.

Despite the split of Tillandsioideae into 4 main lineages, which were considered as separate tribes (Pogospermeae Brongn. (1864) = Catopsideae Harms (1930), Glomeropitcairnieae Harms, Tillandsieae Rchb., Vrieseeae W. Till & Barfuss) in Barfuss & al. (2005) and the transfer of *W. insignis* (Mez) W. Till, Barfuss & Samuel in Barfuss & al. (2004) no further taxonomic conclusions were drawn in any previous phylogenetic study, leaving the most interesting questions unanswered: what are the generic and infrageneric units within Tillandsioideae and how can these be defined genetically *and* morphologically?

Aiming at answering these question we conducted phylogenetic analyses of previously-used plastid and newly-generated nuclear DNA sequences, i.e., (1) plastid *atpB-rbcL*, *matK*, *rbcL*, partial *rbcL-accD*, *rps16* intron, partial *trnK* intron, *trnL* intron, and *trnL-trnF* (taken from Barfuss & al., 2005) and (2) nuclear *PHYC* (phytochrome C) and *PRK* (phosphoribulokinase) together with a re-evaluation of potentially useful morphological characters of the habit and various floral elements.

Materials and Methods

Taxon selection

Plant material was primarily selected based on results of Barfuss & al. (2005) and the taxonomic treatment of Smith & Downs (1977). Ninety-seven accessions out of 122 from the first study together with 347 new accessions were included in the present investigation, making a total of 444 individuals of tillandsioid species. A complete list of all plant material examined including nomenclatoric authors can be found in the supplementary data (Appendix). Sampling within recognized genera, subgenera and sections was significantly increased: *Alcantarea* (23 acc.), *Catopsis* (10 acc.); *Glomeropitcairnia* (3 acc.), *Guzmania* (52 acc.), *Mezobromelia* (7 acc.), *Racinaea* (23 acc.), *Vriesea* sect. *Vriesea* (29 acc.), *V.* sect. *Xiphion* p.p., typo excluso (23 acc.), *Werauhia* sect. *W*. (9 acc.), and *W.* sect. *Jutleya* (7 acc.); the main focus has been within the species-richest genus *Tillandsia* (258 acc.): *T.* subg. *Allardtia* (100 acc.), *T.* subg. *Anoplophytum* (44 acc.), *T.* subg. *Phytarrhiza* (meso- and semi-mesophytic: 31 acc., xerophytic: 10 acc.), *T.* subg. *Pseudalcantarea* (7 acc.), *T.* subg. *Diaphoranthema* (12 acc.), and *T.* subg. *Tillandsia* (54 acc.). Members of *T.* subg. *Pseudalcantarea* and meso- and semi-mesophytic taxa of *T.* subg. *Phytarrhiza* were nearly completely sampled (missing only *T. narthecioides* Presl), to clarify issues of high levels of polyphyly within these subgenera (Gilmartin & Brown, 1986; Beaman & Judd, 1996; Till, 2000b), and to test for either an early split of species from the rest of *Tillandsia* (*T.* subg. *Pseudalcantarea*) or affinities to the genus *Racinaea* (meso- and semi-mesophytic *T.* subg. *Phytarrhiza*) based on earlier plastid DNA results (Barfuss & al., 2005). Additional tillandsioid taxa were chosen to include species with a distinct morphology, which

indicates an inadequate generic, subgeneric or sectional placement by Smith & Downs (1977), an ancestral position within recognized taxa or probable separate phylogenetic units within Tillandsioideae, e.g., the large, broad-leaved and water-impounding or three-pinnate species of *T.* subg. *Tillandsia* occurring in north-western South America (e.g., Till, 2000b; León & Sagástegui, 2008), the former xerophytic, grey-leaved *Vriesea* species (e.g., Grant, 1993b), or the *Tillandsia tectorum* complex (e.g., Hromadnik, 2005) which is associated with the *Tillandsia plumosa* complex (= *Viridantha*) in an earlier study (Barfuss & al., 2005). As an outgroup accessions of *Glomeropitcairnia* and *Catopsis* were used, since in all previous investigations these genera came out as two early diverging lineages, both being sister to each other and together being sister to the core Tillandsioideae. Relevant type species for genera and infrageneric units were investigated for nearly all taxa except *Racinaea* and *Cipuropsis*, i.e., *R. cuspidata* (L.B. Sm.) M.A. Spencer & L.B. Sm. and *Ci. subandina* Ule (syn. *V. subandina* (Ule) L.B. Sm. & Pittendr.), respectively.

Most material came from the botanical garden of the University of Vienna and the private collection of H. & L. Hromadnik. Additional samples were donated by the botanical gardens of Berlin-Dahlem, Göttingen, Heidelberg, Kew, München, Linz, and Sarasota and the private collections of W. Adlassnig, R. Ehlers, P. Lechner, E.M.C. Leme, J.M. Manzanares, J.P. Pinzon, M. Speckmaier and M. Winkler. Some accessions were also collected by the first and/or second author during scientific fieldtrips and/or student expeditions to Venezuela (2000), the Dominican Republic (2001, 2002), and Ecuador (2003, 2004, 2006). Species nomenclature and infrageneric classification basically follows Smith & Downs (1977) with alterations by Luther & Sieff (1994, 1997), Luther (2001, 2008, 2010), Luther & Rabinowitz (2010) and Grant (1993b, 1994b, 1995b, 2005). Additional taxa accepted, but classified differently or missing in the taxonomic literature followed are *Guzmania fusispica* Mez & Sodiro [≠ *G. osyana* (E. Morren) Mez], *Tillandsia buseri* var. *nubicola* Gilmartin [≠ *T. buseri* Mez], *T. fosteri* Gilmartin [≠ *T. demissa* L.B. Sm.], *T. macropetala* Wawra [≠ *T. viridiflora* (Beer) Baker], *T. malzinei* (E. Morren) Baker [= *Vriesea malzinei* E. Morren], *T. recurvifolia* var. *subsecundifolia* (W. Weber & Ehlers) W. Till [≠ *T. leonamiana* E. Pereira], and the recently described new species *Racinaea tillii* Manzan. & Gouda (Manzanares & Gouda, 2010). However, from the results chapter onwards the new nomenclature is used, which is presented in the chapter "Classification of Tillandsioideae" and can be visualized in Figure 2 (see Appendix) and Tables 4 and 5.

Gene selection

Data from seven plastid DNA regions were taken from a preceding study (Barfuss & al., 2005). No additional plastid sequences were generated, because the original sampling already covered most of the accepted taxonomic lineages and preference was given to new nuclear DNA sequences. Eight nuclear gene regions were initially screened for a subset of Tillandsioideae accessions used in Barfuss & al. (2005) to select appropriate genomic regions for sequencing the more exhaustive sample set. DNA regions surveyed included the multi-copy internal transcribed spacer of the nuclear ribosomal DNA (ITS nrDNA; e.g., Chew & al., 2010), and the low-copy nuclear genes glucose-6-phosphate isomerase, cytosolic (*PGIC*; e.g., Ford & al., 2006), malate synthase (*MS*; e.g., Lewis & Doyle, 2001, 2002), nitrate reductase (*NIA*: e.g., Howarth & Baum, 2002), phosphoribulokinase (*PRK*; e.g., Lewis & Doyle, 2002; Thomas & al., 2006), phytochrome C (*PHYC*; e.g., Mathews & Donoghue, 1999; Samuel & al., 2005), *RPB2* (RNA polymerase II, beta subunit; e.g., Denton & al., 1998; Oxelman & al., 2004), and xanthine dehydrogenase (*XDH*; e.g., Górniak & al., 2010). Parameters for the selection of effective DNA regions

were (1) the number of loci obtained, (2) the performance of primers and PCR conditions for both amplification and sequencing, (3) the occurrence of homopolymers and microsatellites, which are often hard to sequence, (4) the length of the PCR products and (5) the number of potentially phylogenetically informative characters. The primary target was to obtain one or more genomic regions which are either well homogenized (ITS nrDNA) or effectively single-copy (other nuclear regions) to be mostly sequenced directly. *PHYC* and *PRK* were finally selected for phylogenetic analyses (see "Results" for the reasons and chapter 3). All nuclear sequences where generated newly for this study and have been archived in NCBI's GenBank (http://www.ncbi.nlm.nih.gov/genbank).

Molecular data and analyses

Total DNA extractions were done from fresh or silicagel-dried material mainly following a sorbitol/CTAB-based method for difficult plant tissue (Tel-Zur & al., 1999) with modifications according to Russell & al. (2010a). In some cases the 2x CTAB procedure described by Doyle & Doyle (1987) adopted for mini columns, the DNeasy® Plant Mini Kit (QIAGEN®) following the manufacturers protocol, or a combination of both were used; details for the latter can be requested from the first author. Initial primer sequences and PCR conditions for *PRK* and *PHYC* were described by Schulte & al. (2009) and Russell & al. (2010b), respectively. Details for general PCR and sequencing reactions, occasional cloning and other laboratory materials and methods followed chapter 3 of this thesis. Both the *PHYC* and *PRK* genes were amplified and sequenced using sometimes slightly modified or additional primers. Details of all primers used are given in Tables 1 (*PHYC*) and 2 (*PRK*) (see also chapter 3 for more primer details).

Raw sequences were initially analysed and edited using the Sequencing Analysis Software v5.3 (Applied Biosystems®, Life Technologies™ Corporation). A contig of forward and reverse DNA strands from both PCR and/or internal sequencing primers was generated with the SeqMan Pro module of the Lasergene® v8.1 software package (DNASTAR, Inc.). Sequences were edited and a consensus was exported as text file in fasta format. Usually fasta files were pooled together and aligned initially with MUSCLE v3.8 (Edgar, 2004a, b) and then adjusted by eye with the BioEdit v7.0.5 (Hall, 1999) following the guidelines of Kelchner (2000) and Borsch & Quandt (2009). In very polymorphic DNA regions containing numerous indels related taxa were aligned first. Relationships were pre-estimated from previous results or preliminary phylogenetic analyses. Only then were individual alignments aligned together using the profile alignment function of MUSCLE and adjusted by eye using BioEdit. Aligned matrices can be obtained from the first author upon request.

When allelic length variation between priming sites was observed, editing of sequences coming from both directions was not straightforward and done in such a way that the missing DNA sequence parts of one allele (largely small inserted repeats or deletions) were ignored and allelic consensus sequences were used further. This was accomplished by identifying the type of indel and extracting the longer allele from shifted electropherograms, which are a result of non-homologous parts of the two different alleles to be overlaid. If the number of indels was too high with two or more indels between priming sites in both alleles, editing of sequences became difficult and doubtful, so that in such cases PCR products were reinvestigated with a cloning step included. Edited indel containing consensus sequences were only included for analyses, when prior evaluation steps on polymorphic sites (see below) indicated that allelic indels are also most likely due to variation within a single species or within a closely related species complex.

Table 1. Primers used for *PHYC* with their location given according to base positions in exon 1 of the *PHYC* reference sequence of *Oryza sativa* (AF141942).

name	sequence (5'–3')	position (direction)	comments	reference
phyc515f-br	AAGCCCTTYTACGCTATCCTGCACCG	490–515 (up)	Bromeliad-specific PCR primer	This study
phyc524f-br	GCTATCCTGCACCGGATCGAYGT	502–524 (up)	Bromeliad-specific internal sequencing primer	This study
phyc974f-br	GCTCCTCACGGCTGCCACGCTCA	952–974 (up)	Bromeliad-specific internal sequencing primer	This study
phyc1145r2-mo	CAACAGGAACTCACAAGCATATC	1,167–1,145 (down)	monocot-specific internal sequencing primer	This study
phyc1690r-br	TCAACATCTTCCCAYGGGAGGCT	1,712–1,690 (down)	Bromeliad-specific internal sequencing primer	This study
phyc1699r-br	ATWGCATCCATTTCAACATCTTCCCA	1,724–1,699 (down)	Bromeliad-specific PCR primer	This study

Table 2. Primers used for *PRK* with their location given according to base positions in exon regions of the *PRK* reference sequence of *Oryza sativa* (NM_001054360); exon 1 (1–545), exon 2 (546–630), exon 3 (631–715), exon 4 (716–960), exon 5 (961–1,212).Bold italicized numbers denote positions where primers are located partly within intron parts of *PRK*.

name	sequence (5'–3')	position (direction)	comment	reference
prk621f	TCAGCAATGAGGTTAAATTTGCATGG	exon 2, 596–621 (up)	Bromeliad-specific PCR primer	This study
prk630f	AAATTTGCATGGAAAATTCAGGTC	exon 2, intron 2, 610–*633* (up)	Bromeliad-specific internal sequencing primer	This study
prk734f	CTGCAGATCCGCAGAAGAAATATGC	intron 3, exon 4, *710*–735 (up)	Bromeliad-specific internal sequencing primer	Schulte & al., 2009
prk890r	GGGTATGAGCATGTCAATTTCCTCC	exon 4, 914–890 (down)	Bromeliad-specific internal sequencing primer	This study
prk1057r	CTTCAGCATTTGTTGTGTCACCTC	exon 5, 1,080–1,057 (down)	Bromeliad-specific internal sequencing primer	This study
prk1069r2	GGAAAATCTGCRTGCTTCAGCATTTG	exon 5, 1,094–1,069 (down)	Bromeliad-specific PCR primer	This study

Table 3. Attributes of analyzed matrices of the five datasets D1–5 and parsimony scores of equally most parsimonious trees after analysis using PAUP*. The sequence length is given for individual markers only. bp = base pairs, var. char. = variable characters, PIC = parsimony informative characters, CI = consistency index, RI = retention index.

matrix	lenght [bp]	no. of char.	no. of var. char.	no. of PICs	no. of trees	tree length	CI	RI
plastid DNA (D1)		6,115	744 (12%)	378 (6%)	>100,000	1,210	0.685	0.831
PHYC (D2)	1,159–1,192	1,228	485 (39%)	369 (30%)	>100,000	1,398	0.473	0.867
PRK (D3)	831–1,692	3,291	737 (22%)	534 (16%)	>100,000	2,232	0.478	0.870
PHYC + *PRK* (D4)		4,519	1,222 (27%)	903 (20%)	>100,000	3,775	0.458	0.859
PHYC + *PRK* + plastid DNA (D5)		10 634	1,966 (18%)	1,297 (12%)	>100,000	5,043	0.507	0.853

Table 4. Accepted tribal, subtribal and generic concepts and informal clades of Tillandsioideae (Bromeliaceae) in comparison to earlier classification systems (based on the placement of the nomenclatoric types). i.s. = incertae sedis.

Smith & Downs (1977)	**Smith & Till (1998)**	**Barfuss & al. (2005)**		**Barfuss, Till & Samuel**				
genus	**genus**	**genus**	**tribe**	**genus/subtribe/clade**			**tribe/clade**	
Glomeropitcairnia	*Glomeropitcairnia*	*Glomeropitcairnia*	Glomeropitcairnieae	*Glomeropitcairnia*			Glomeropitcairnieae	
Catopsis	*Catopsis*	*Catopsis*	Catopsideae	*Catopsis*			Pogospermeae	
Vriesea	*Alcantarea*	*Alcantarea*	Vrieseeae	*Alcantarea*		Vrieseinae	Vrieseeae	Core Tillandsioideae
	Vriesea	*Vriesea*		*Vriesea*				
	Werauhia	*Werauhia*		*Werauhia*		Cipuropsidinae		
	Vriesea	*Vriesea*		*Cipuropsis*	*Cipuropsis-Mezobromelia* clade			
				Splendens clade				
				Chrysostachys clade				
				Vriesea tuerckheimii (i.s.)				
Mezobromelia	*Mezobromelia*	*Mezobromelia*	Tillandsieae	*Mezobromelia*				
Tillandsia	*Tillandsia*	*Tillandsia*		*Singularis* clade				
				Josemania		Core Tillandsieae	Tillandsieae	
				Lemeltonia				
				Rothowia				
				Tillandsia				
		Viridantha						
	Racinaea	*Racinaea*		*Racinaea*				
Guzmania	*Guzmania*	*Guzmania*		*Guzmania*				

Table 5. Accepted subgeneric concept, informal clades, and unclassified species of *Tillandsia* (Bromeliaceae) and recent generic segregates in comparison to earlier classification systems (based on the placement of the nomenclatoric type). A dashed line indicates further informal subdivision of a given taxon. subg. = subgenus, p.p. = pro parte, i.s. = incertae sedis.

Smith & Downs (1977)	**Till (2000)**	**Barfuss & al. (2005)**	**Barfuss, Till & Samuel**
Tillandsia subg. *Pseudalcantarea*	*Tillandsia* subg. *Pseudalcantarea*	*Tillandsia* subg. *Pseudalcantarea*	*Tillandsia* subg. *Pseudalcantarea*
Vriesea p.p., typo excluso (xerophytic, grey-leaved)	*Tillandsia* subg. *Tillandsia*	*Tillandsia* subg. *Tillandsia*	*Tillandsia* subg. *Pseudovriesea*
Tillandsia subg. *Tillandsia*			*Rauhii* clade
			Tillandsia subg. *Tillandsia* (syn. *Tillandsia* subg. *Allardtia*)
Tillandsia subg. *Allardtia*	*Tillandsia* subg. *Allardtia*	*Viridantha*	*Tillandsia* subg. *Viridantha*
		Tillandsia subg. *Allardtia* p.p. majore, typo excluso	*Biflora* clade
			Tillandsia australis (i.s.)
			Tillandsia edithae (i.s.)
			Tillandsia disticha (i.s.)
			Tillandsia pseudomicans (i.s.)
			Tillandsia sphaerocephala (i.s.)
Tillandsia subg. *Anoplophytum*		*Tillandsia* subg. *Anoplophytum*	*Xiphioides* clade
			Tillandsia esseriana (i.s.)
	Tillandsia subg. *Anoplophytum*		*Tillandsia* subg. *Anoplophytum*
			Gardneri clade
			Tillandsia albertiana (i.s.)
			Tillandsia nana (i.s.)
Tillandsia subg. *Diaphoranthema*	*Tillandsia* subg. *Diaphoranthema*	*Tillandsia* subg. *Diaphoranthema*	*Tillandsia* subg. *Diaphoranthema*
Tillandsia subg. *Phytarrhiza*	*Tillandsia* subg. *Phytarrhiza* (xerophytic)	*Tillandsia* subg. *Phytarrhiza* (xerophytic)	*Tillandsia* subg. *Phytarrhiza*
			Purpurea clade
	Tillandsia subg. *Phytarrhiza* (meso-/semi-mesophytic)	*Tillandsia* subg. *Phytarrhiza* (meso-/semi-mesophytic)	*Josemania*
			Lemeltonia
			Rothowia
			Racinaea subg. *Pseudophytarrhiza*
Tillandsia subg. *Pseudo-Catopsis*	*Racinaea*	*Racinaea*	*Racinaea* subg. *Racinaea*

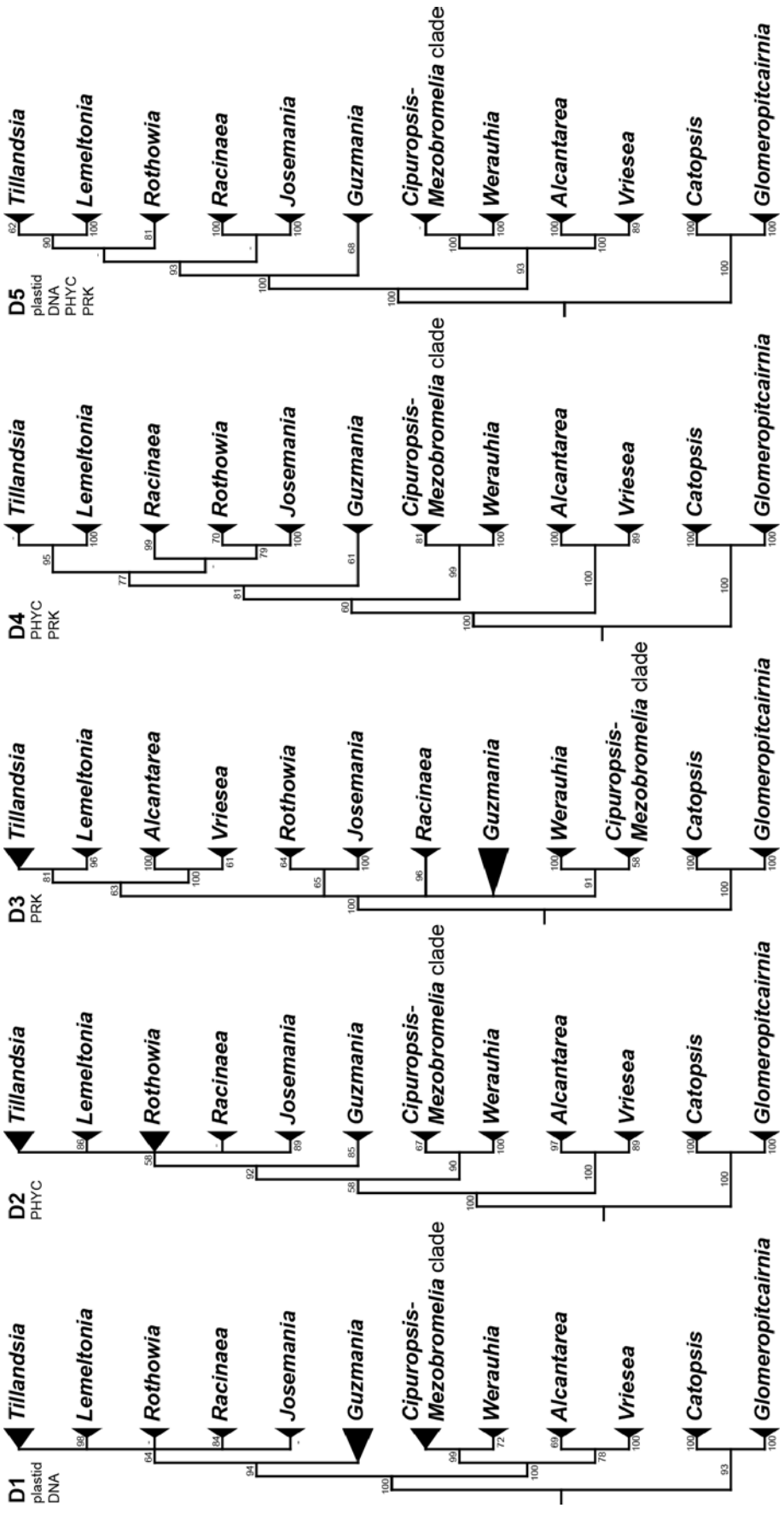

Figure 1. Simplified strict consensus trees of equally most-parsimonious trees found in parsimony analyses of individual datasets (D1–D5) supplemented with BP from parsimony bootstrap analysis either above or below branches; triangles sitting in terminal position on a branch indicate holophyletic units, whereas triangles emerging from within a node indicate several branches of that unit on a polytomy. Only relationships of all except two recognized genera and one informal clade are displayed. *Cipuropsis* and *Mezobromelia* are included in the *Cipuropsis-Mezobromelia* clade including informal clades. D1 = plastid DNA, D2 = *PHYC*, D3 = *PRK*, D4 = *PHYC* + *PRK*, D5 = plastid DNA (+ *PHYC* + *PRK*.

Nuclear alignments were evaluated prior to phylogenetic analyses for the impact of polymorphic sites (Single Nucleotide Polymorphism = SNP). The need for these additional steps before phylogenetic analyses has been pointed out by Jabaily & Sytsma (2010) and Russell & al. (2010b) in detail. The occurrence of SNPs in resulting electropherograms of nuclear allelic consensus sequences was inferred as allelic variation within the given sample. In the final alignments these positions were carefully evaluated to see if their patterns suggest the presence of similar alleles in more distantly related taxa indicating polyploidization, hybridization, reticulation, introgression events or the occurrence of ancient alleles (incomplete lineage sorting).

The aligned plastid DNA matrix with some taxa deleted (because of missing nuclear DNA sequence information) was taken from Barfuss & al. (2005). A 25 bp region from *atpB-rbcL* (a partly inverted composed poly-A/poly-T region) where homology could not be unambiguously assessed, was excluded here from the analyses (positions 4,321–4,345 of the plastid DNA dataset and positions 8,840–8,864 of the combined dataset, respectively). Five datasets were analyzed, i.e., (D1) plastid DNA, (D2) *PHYC*, (D3) *PRK*, (D4) *PHYC* + *PRK*, and (D5) *PHYC* + *PRK* + plastid DNA, and compared with results from Barfuss & al. (2005). In four cases sequences of the plastid DNA dataset had to be combined with nuclear sequences from a different source of the same species (*G. variegata* L.B. Sm., *T. barclayana* Baker, *T. lindenii* Regel, *T. stricta* Sol. ex Sims), since the original sample of Barfuss & al. (2005) was not available anymore. These species could not be excluded because they represent genetic entities that would otherwise be either underrepresented or non-existent in the combined dataset. Alleles resulting from cloned nuclear sequences were combined with duplicated plastid DNA sequences only when their position in individual nuclear trees did not significantly contradict the plastid DNA tree topologies.

Analyses of the aligned and evaluated individual and combined datasets included maximum parsimony (MP) and Bayesian metropolis-coupled MCMC inference (BI). MP analyses were implemented in the program PAUP* 4.0b10 (Swofford, 2003) using a two-step heuristic search strategy due to the complexity of DNA sequence data. The first step of heuristic searches was done with 1,000 random sequence additions, TBR branch swapping and holding 10 trees each step to reduce time spent swapping on non-optimal trees, and saving always 100 shortest trees per replicate, even if the trees were not the shortest over all replicates (to check for multiple island in the resulting tree space). In the second round trees obtained from the first analyses were taken as starting trees and heuristic searches were conducted until all or a maximum of 100,000 shortest trees were saved, but the tree search was allowed to continue to swap on all input trees for shorter ones (swapping to completion). Gaps were always treated as missing characters and no gap coding procedure was applied, mainly because gaps resulting from allelic length variation in the very indel-rich *PRK* dataset (D3) were ignored.

BI analyses were done with the program MrBayes 3.1 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003) using the MPI version (Altekar & al., 2004) at the University of Oslo Bioportal (http://www.bioportal.uio.no/). Best-fitting nucleotide substitution models were determined in advance using MrModeltest 2.3 (Nylander, 2004) following the Akaike information criterion. Although the best model for each dataset was determined as GTR + I + G, analyses of the two combined datasets D4 and D5 were still partitioned (*PHYC*, *PRK*, plastid DNA) to allow different parameters for each individual region to be estimated separately. Four independent runs of four MCMC chains were done for ten million generations, sampling every 1,000 generations and with a burn-in of 8 to 25 %, which was evaluated for each run individually. Other parameters and priors were left as default.

Table 6. Morphological characters used to differentiate among genera and informal clades of Tillandsioideae. Taxa are arranged according to the "Classification of Tillandsioideae" chapter. Values for the septal nectary ducts, endostome type, and embryo type were exclusively taken from literature. m = mesophytic, sm = semi-mesophytic, sx = semi-xerophytic, x = xerophytic; f = free, c = connate/conglutinate; p = present, a = absent; ap = appendage, ov = ovule, pp = pseudopappus; cb = convolute-blade, ce = conduplicate-erect, cf = coralliform, cp = conduplicate-patent, cs = conduplicate-spiral, cup = cupulate, cupp = cupulate-papillate, se = simple-erect, tl = tubolaciniate, pi = pinnatisect; ? = missing data; (?) = information questionable in original source; data in () = rarely.

Genus/clade	Habit	Petals	Petal appendages	Filaments	Petal-filament	Anthers	Aperture type	Ovary position	Ovule appendage (chalazal)	Setal nectary ducts	Stigma type	Pseudopappus	Endostome type	Embryo type
Glomeropitcairnia	m	f	p	f	f	f	diffuse	½–⅔ inferior	p: ap > ov	?	cb	micropylar	?	?
Catopsis	m	f	a	f	f	f	*Catopsis* type	superior–⅛ inferior	pp	ascending	se	chalazal	d	g
Guzmania	m	c	a / (p)	f	c / (f)	f / (c)	diffuse / inaperturate	max. ⅓ inferior	a	horizontal	se / cb	micropylar	a / b / (e)	a / (b)
Josemania	m / sm	f	a	f	f	f	insulae	max. ⅓ inferior	a	?	pi	micropylar	?	?
Lemeltonia	sm / (m)	f	a	c	f	f	insulae	max. ⅓ inferior	a	horizontal	cf	micropylar	d	b
Racinaea	m	f	a	f	f	f	insulae to diffuse	max. ⅓ inferior	a	horizontal	se / (slightly cs) / (cf)	micropylar	c / d / f (?)	b
Rothowia	m	f	a	f	f	f	insulae to diffuse	max. ⅓ inferior	a	?	cb	micropylar	e	b / (–a)
Tillandsia	m / (sm) / (sx) / x	f	a / (p)	f	f	f	operculum / diffuse, insulae, Alcantarea type	max. ⅓ inferior	p: ap ≤ ov or (ap > ov) / a	horizontal	se/ cs / (cb) / (cp)	micropylar	e / f / g / h / i / k / l / m / n	b / c / d / e / f / (–g)
Cipuropsis	m	c (ca. ¼)	p	f	c (ca. ¼)	f	diffuse to insulae	max. ⅓ inferior	a	?	se	micropylar	c	a
Mezobromelia	m	c (ca. ½)	p	f	c (ca. ½)	c	diffuse / inaperturate	max. ⅓ inferior	a	?	se	micropylar	?	?
Werauhia	m	f	p / (a)	f	f	f	insulae	max. ¼ inferior	a	descending	cup	micropylar	d	a / (b)
Chrysostachys clade	m	c (ca. ¼)	p	f	c (ca. ¼)	?	?	max. ⅓ inferior	a	?	se	micropylar	?	?
Singularis clade	m	c (ca. ¼)	a	f	c (ca. ¼)	f	fine insulae	max. ⅓ inferior	a	?	se	micropylar	?	?
Splendens clade	m	f	p	f	f	f	?	max. ⅓ inferior	a	?	cs	micropylar	c / d	a
V. tuerckheimii	m	f	p	f	c (ca. ¼)	f	?	max. ⅓ inferior	p: ap ≤ ov	?	cupp	micropylar	?	?
Alcantarea	m / (sm)	f	p	f	f	f	*Alcantarea* type	max. ⅓ inferior	p: ap > ov	?	cp / (ce)	micropylar	?	?
Vriesea	m / (sm) / (sx)	f / (c) short	p	f	f / (c) short	f	insulae	max. ⅓ inferior	p: ap ≤ ov / (a)	descending	cb / tl	micropylar	e / (–c)	b / (a)

Table 7. Morphological characters used to differentiate among subgenera and informal clades of *Tillandsia*. Taxa are arranged according to the "Classification of Tillandsioideae" chapter. m = mesophytic, sm = semi-mesophytic, sx = semi-xerophytic, x = xerophytic; p = present, a = absent; ap = appendage, ov = ovule, cb = convolute-blade, ce = conduplicate-erect, cp = conduplicate-patent, cs = conduplicate-spiral, se = simple-erect; data in () = rarely.

Subgenus/clade	Habit	Petal shape	Petal appendages	Ovule appendage (chalazal)	Stigma type	Endostom type	Embryo type
Tillandsia subg. *Anoplophytum*	(sx) / x	lingulate	a	a / p: ap ≤ ov	se / cs	m	(e–f) / f
Tillandsia subg. *Diaphoranthema*	x	lingulate / unguiculate	a	p: ap ≤ ov	se	m/n	e
Tillandsia subg. *Phytarrhiza*	x	unguiculate	a	p: ap ≤ ov	se	m	e / f
Tillandsia subg. *Pseudalcantarea*	m	lingulate	a	a	(cs) / cp	f	b
Tillandsia subg. *Pseudovriesea*	sx / (x)	lingulate	p / (a)	p: ap ≤ ov	cs / ce	f	(b–) / ± f
Tillandsia subg. *Tillandsia*	m / sm / sx / x	lingulate	a / (p)	p: ap ≤ ov / ap > ov	cs / (se)	e/g/i/k	b / c / f (–g)
Tillandsia subg. *Viridantha*	x	lingulate	a	a / p: ap ≤ ov	se / cb	h/l	c / f
Biflora clade	m / sm / (sx)	lingulate	a	a / p: ap ≤ ov	cs / (se)	e/g/h	b / f
Gardneri clade	x	lingulate	a	a / (p: ap ≤ ov)	se	m	(e–f) / f
Purpurea clade	x	unguiculate	a	a / (p: ap ≤ ov)	cs	h/l	c–f
Rauhii clade	m	lingulate	a	p: a ≤ o/(ap > ov)	cs / (cp)	h	d
Xiphioides clade	x	unguiculate	a	p: ap ≤ ov	se / (cs)	m	f

Support was obtained from 50 % Bayesian majority rule consensus trees (Posterior Probabilities, PP) and from parsimony Bootstrap analyses done in PAUP* 4.0b10. Bootstrap percentages (BPs) were calculated using 1,000 replicates, TBR branch swapping, simple sequence addition, holding 10 trees each step and saving 100 shortest trees per bootstrap replicate.

Combinability of plastid DNA, *PHYC* and *PRK* datasets was determined by visual inspection of individual bootstrap consensus trees (Whitten & al., 2000). Measures of incongruence like the incongruence length difference test (ILD; Farris & al, 1994, 1995; Lee, 2001) have been demonstrated not always to be useful indicators of data combinability, specifically in the light of relatively recently divergent plant groups with a lower sequence divergence in plastid DNA compared to nuclear DNA (Yoder & al., 2001; Reeves & al., 2001). We use the following descriptions for categories of BP: unsupported, <50%; weak, 50–79%; moderate, 80–89%; strong, 90–100%. In the case of only weakly- to moderately-supported incongruent topologies between the individual trees, direct combination was regarded as appropriate. The artifact of long-branch attraction (LBA) can sometimes yield high moderate or strong BP for a phylogenetically wrong clade, therefore the number of autapomorphies for and the taxon sampling within the specific clades are also considered (Bergsten, 2005).

Morphological data and analyses

Morphological characters were collected and documented over several years from herbarium sheets, liquid flower preservations, fresh material cultivated in the botanical garden of Vienna or from several donors (see "Taxon selection") using a stereo microscope (Olympus SZ60 Stereo Zoom Microscope) or SEM technology (either with a JEOL T-300 or JEOL JSM-6390 Scanning Electron Microscope). Characters of some taxa were extracted and carefully evaluated from the original description of the respective taxon, the last monograph (Smith & Downs, 1977) or selected literature (e.g., Brown & Gilmartin 1984, 1989b; Böhme, 1988; Groß, 1988; Brown & Terry, 1992; Halbritter, 1992). Except for the general habit, the gathered morphological information mainly comprised characters of diverse floral elements like sepals, petals (incl. petal appendages), stamens (incl. filaments, anthers and pollen), carpels (incl. stigma and ovules) as well as ovary position and seed morphology (Tables 6, 7). Characters were manually mapped on resulting phylogenetic trees and empirically useful morphological traits taken for the circumscription of obtained clades. No phylogenetic analysis of a morphological data matrix was performed, because of missing data in many of accessions studied. Although several species could not be investigated for all used morphological traits, it was assumed that characteristics can be applied to higher taxonomic ranks.

Results

Table 3 provides the attributes of the analyzed matrices, together with the details of the equally-most-parsimonious trees found in parsimony analyses of each of the five datasets. Tables 6 and 7 summarize morphological traits found useful so far and used to differentiate between genera, subgenera and informal clades. Simplified strict consensus trees of shortest trees from parsimony analyses supplemented with BP are shown in Figure 1, D1–5 for comparing main differences in tree topologies between each dataset. One most parsimonious phylogram (Figure 2, see Appendix) from total combined analysis that reflects our understanding on phylogenetic relationships best was chosen and supplemented with BP and PP. Alternative topologies of terminal branches and backbone relationships from Bayesian analysis are displayed in boxes

mostly in front of the corresponding clades (terminals) or on the upper-left side (backbones) of Figure 2. Their positions within the parsimony tree are highlighted by capital letters (Figure 2, A–P: terminals, Q–S: backbones). Other trees from different analyses (parsimony and Bayesian) of each individual dataset are not shown and can be requested from the first author. Only relationships of genera accepted in the present investigation (except *Cipuropsis* and *Mezobromelia*) and one informal infrasubtribal clade (*Cipuropsis-Mezobromelia* clade) are shown and their results presented for each individual dataset (Figure 1, D1–5). Results including all tribal, subtribal, generic, and infrageneric units and informal infrageneric clades are displayed for the total combined dataset only (Figure 2). The nomenclature, taxonomy and morphological characterization of clades, and the taxonomic position of each species studied can be obtained from the chapter "Classification of Tillandsioideae", Figure 2 or Tables 4 and 5, respectively. Main geographic distributions are given in the key to the genera.

Gene selection

From eight genomic regions screened initially, only *PRK* and *PHYC* were selected because (1) they were easy to amplify and sequence in most cases, (2) had the most variation in relation to their average length, and (3), most importantly, amplifications resulted in single, clear bands. This indicates an effectively single-copy nature of both nuclear regions and therefore allows PCR products to be sequenced directly without cloning, at least in individuals without allelic indel variation or hybrid origin. This is in agreement with previous studies of these genes in other monocot genera like *Puya* Molina (Bromeliaceae: Jabaily & Sytsma, 2010), *Oryza sativa* L. (*Poaceae*: Chen & al., 2004), or *Chamaedorea* Willd. (*Arecaceae*: Thomas & al., 2006). Reports of possible multiple loci (paralogs) of *PRK* in subfamily Bromelioideae (Schulte & al., 2009) cannot be confirmed for Tillandsioideae and are possibly due to a gene duplication event within Bromelioideae (D. Silvestro & K. Schulte, unpubl. data). Within *PRK* introns few polynucleotide runs (SSRs) were observed and could not be avoided (see supplementary data): (1) in intron four a more frequent SSR was detected in most tillandsioid accessions (except for taxa were the SSR containing region has been lost), (2) in intron two few *Guzmania* species exhibit a large insertion containing a second SSR, and (3) in intron three of *G. multiflora* (André) André ex Mez, which also exhibits a larger insertion, a third SSR was observed. The other regions were discarded either because they made problems in amplifications and sequencing (ITS nrDNA), primer combinations only amplified a comparatively short DNA fragment (*RPB2*, *MS*), or sequence variation and taxon sampling was to low (*NIA*, *PGIC*, *XDH*).

Nuclear data evaluation

Details of allelic length variation (heterozygous indels) and polymorphic sites (SNPs) are given separately for each nuclear marker (see below). Evaluation of both *PHYC* and *PRK* data revealed that none of the investigated samples showed evidence for polyploidization, hybridization, reticulation, introgression or incomplete lineage sorting involving more distantly related species. Although polyploidy (either auto- or allopolyploidy) can have an major impact on the allele distribution and frequency within an/all affected species, it can be rejected for most species analyzed here since in most cases allelic variation can be traced back to two alleles (see supplementary data). This is also in agreement with previous findings that most Tillandsioideae species (and Bromeliaceae species in general) show a diploid chromosome number of 2n = 2x = 50 (e.g., Brown & Gilmartin, 1989a; Palma-Silva & al., 2004). In two species within *T.* subg. *Diaphoranthema* (*T. capillaris* Ruiz & Pav., *T. virescens* Ruiz & Pav.), allelic variation of SNPs

supports already documented cases of polyploidy with 2n = 4x = 100 (Till, 1992), either autopolyploidization or allopolyploidization involving closely related species. However, these were ignored since they have no impact on the current results (incl. branching patterns and statistical support) and are insignificant for the current study. A possible case of a hybrid origin involving sister taxa was found in the sample determined as *Ro. platyrhachis*. One allele of this accession is strongly associated with *Ro. wagneriana*. Sequence variation between or within other closely related species in terminal branches was too low to either reject or confirm any of the above mentioned hypotheses. Therefore, these were ignored, since the scope of the present study is to resolve deeper nodes primarily and not individual relationships of recently diverged species. Otherwise it would also be necessary to question and investigate the species concept used involving a very broad sampling and other molecular markers. However, polytomies and less resolved or supported clades at any hierarchical level in resulting trees were primarily caused by a lack of phylogenetic signal rather than conflicting polymorphic sites.

Plastid DNA data and analyses (dataset D1)

Barfuss & al. (2005) already explored attributes and detailed results from the plastid DNA data analyses. A summary of generic relationships based on a strict consensus tree of a maximum parsimony analysis supplied with BP is given in Figure 1, D1. Differences in the attributes between the first plastid DNA study and the present investigation (Table 3) are due to the deletion of several taxa from the plastid DNA matrix, where no nuclear DNA data was available, and the exclusion of a 25 bp region from *atpB-rbcL*. Deleting these taxa and base pairs had no significant effect on the topology of obtained trees, identical to those of Barfuss & al. (2005).

PHYC data and analyses (dataset D2)

Characteristics of the analyzed *PHYC* matrix are given in Table 3. PCR primers amplified fragments of partial exon one ranging from 1,159 (*Werauhia insignis*) to 1,192 bp (*Guzmania acorifolia* (Griseb.) Mez, *G. condensata* Mez & Wercklé), but mostly a fragment of 1,177 bp. According to the tree topology, the latter size can be inferred as the ancestral state. Indel events are rare and derived, and present either in single species or few closely related species in terminal branches. Indication of allelic length variation within individuals caused by heterozygous indels was not found in any of the sampled taxa, since in *PHYC* only conserved parts of the exon 1 were amplified. Allelic nucleotide variation as indicated by obvious double peaks at a few positions was found in 220 taxa out of 444 sampled individuals. 224 samples were found to be homozygous for the region sequenced. The number of SNPs in a given heterozygous taxon ranged from 1–15 (the latter seen in *T.* aff. *cucaensis* Wittm. B0735). Distribution of polymorphic sites was evenly across the whole range of *PHYC* with no particular preference for a specific gene region, but significantly higher at the 3rd codon position caused mostly by synonymous substitutions. Evaluation of the effects of SNPs in *PHYC* data indicate that there is not a strong phylogenetic bias in not having allelic data and allelic consensus sequences can be used. We therefore only used the polymorphism-coded *PHYC* dataset for all analyses, with the exception of three taxa, which were cloned for *PRK* and therefore also for *PHYC*, to be able to combine corresponding allelic sequences.

Figure 1, D2 is the strict consensus tree of a MP analysis with BP, which summarizes the generic relationships based on *PHYC* sequence data. As in plastid DNA tree, both *Glomeropitcairnia* (tribe Glomeropitcairnieae) and *Catopsis* (tribe Pogospermeae) are strongly supported (Bootstrap percentage = BP 100) and sisters (BP 100), with the latter clade sister to a strongly-

supported core Tillandsioideae (BP 100). Tribe Vrieseeae is paraphyletic in *PHYC* results, but the resulting grade of subtribes Vrieseinae and Cipuropsidinae has only a weak BP (58). *Vriesea* is holophyletic (BP 89) only when Andean members located in the *Cipuropsis-Mezobromelia* clade, *T. malzinei* and former xerophytic gray-leaved members, the latter two now placed within *Tillandsia*, are excluded. The sister group relationship of *Vriesea* and *Alcantarea* (= subtribe Vrieseinae) is strongly supported (BP 100), as is the holophyletic origin of *Alcantarea* (BP 97). Subtribe Cipuropsidinae is holophyletic (BP 90) and splits into two lineages, *Werauhia* (BP 100) and the *Cipuropsis-Mezobromelia* clade (BP 67). *Mezobromelia* is a weakly-supported holophyletic lineage (BP 57) nested within the *Cipuropsis-Mezobromelia* clade (not shown), which in addition contains taxa previously assigned to *Vriesea* and *Tillandsia*. Tribe Tillandsieae is strongly supported (BP 92). The first lineage which splits from core Tillandsieae is *Guzmania* (BP 85), with *G. hutchisonii* (syn. *M. hutchisonii*) nested inside (not shown). Core Tillandsieae is only weakly supported (BP 58) and relationships of genera are unresolved. Holophyletic taxa are *Josemania* (BP 89), *Racinaea* (no BP) and *Lemeltonia* (BP 86), but all three are placed on a polytomy with different clades of *Tillandsia* and *Rothowia*.

PRK data and analyses (dataset D3)

Attributes of the analyzed *PRK* matrix are given in Table 3. PCR primers amplified fragments ranging from 831 (*G. patula* Mez & Wercklé) to 1,692 bp (*T. ionochroma* André ex Mez), with a mean of 1,086 bp. Indel events are common at all hierarchical levels of obtained trees, both between individuals of a single species and different higher clades. Unlike *PHYC*, evidence of allelic length variation within individuals was detected in 146 of sampled taxa, since the amplified region covered three less conserved introns and four exons (exon 2 to exon 5), with the second and the fifth exon being sequenced only partially. The number of heterozygous indels ranged from 1 to 5 in a given taxon. Indels were only found in the less conserved intron regions and never in the *PRK* exons. Three taxa had to be cloned, since indel variation between alleles was too high to perform unambiguous editing, i.e., *V. psittacina* (Hook.) Lindl., *G. graminifolia* (André ex Baker) L.B. Sm., and *Ro. platyrhachis*. Some others were occasionally cloned to verify allele length (see supplementary data). Allelic nucleotide variation was found in 241 taxa out of 444 sampled individuals. Only 193 samples were found to be homozygous for the whole region sequenced concerning indels and SNPs, which seems to be due to the relatively high rate of evolution in intron sequences. The number of SNPs in a given heterozygous taxon ranged from 1 to 18 (the latter seen in *T. fasciculata* Sw. B0076). Polymorphic sites were primarily found in the less conserved intron parts of the gene and less frequent in exons with mostly synonymous substitutions. Like in *PHYC*, evaluation of the effects of SNPs in *PRK* data indicate that there is not a strong phylogenetic bias in not having allelic data and allelic consensus sequences can be used. We therefore also only use the polymorphism-coded *PRK* dataset for all analyses, with the exception of the three cloned taxa.

Generic relationships based on *PRK* sequence data are shown in Figure 1, D3, which is the strict consensus tree of a MP analysis supplemented with BP. Backbone relationships are much less resolved than in the *PHYC* tree. Topologically identical are the sister group relationship (BP 100) of *Glomeropitcairnia* (BP 100) and *Catopsis* (BP 100) and core Tillandsioideae (BP 100). Subtribes Cipuropsidinae and Vrieseinae are strongly supported (BP 91 and 100, respectively), whereas tribes Vrieseeae and Tillandsieae as well as core Tillandsieae are not supported. *Werauhia* is strongly supported by *PRK* sequence data (BP 100), whereas the *Cipuropsis-Mezobromelia* clade has only weak BP (BP 58). The same picture is seen for *Alcantarea* (BP

100) and *Vriesea* (BP 61), respectively. The relationship of *Vriesea* and *Alcantarea* to a clade containing *Tillandsia* and *Lemeltonia* is surprising, but this is only weakly supported (BP 63). *Guzmania* is not supported and three different clades of *Guzmania* species (not shown) occur in a polytomy with several other clades. *Rothowia* and *Josemania* are resolved as sisters (BP 65), both also being holophyletic (BP 64 and 100, respectively). *Racinaea* is strongly supported (BP 96), but relationships to other genera remain unresolved. *Tillandsia* and *Lemeltonia* are resolved as sister taxa with a moderate BP (BP 81) with *Lemeltonia* being strongly supported (BP 96), but in a polytomy with several other clades of *Tillandsia* species.

Combined nuclear data (dataset D4)

Analyses of concatenated nuclear datasets D2 and D3 (Figure 1, D4) combine tree topologies obtained from individual analyses of *PHYC* and *PRK* (Figure 1, D2–3). Most accepted infrasubfamilial clades and genera are holophyletic with mostly moderate or high BP, i.e., *Glomeropitcairnia* (BP 100), *Catopsis* (BP 100), core Tillandsioideae (BP 100), Vrieseinae (BP 100), *Vriesea* (BP 89), *Alcantarea* (BP 100), Cipuropsidinae (BP 99), *Werauhia* (BP 100), *Cipuropsis-Mezobromelia* clade (BP 81), Tillandsieae (BP 81), core Tillandsieae (BP 77), a sister relationship of *Josemania* and *Rothowia* (BP 79), *Josemania* (BP 100), *Racinaea* (BP 99), *Lemeltonia* (BP 100), and the sister relationship of *Lemeltonia* and *Tillandsia* (BP 95). The grade of paraphyletic Vrieseeae taxa (BP 60), *Guzmania* (BP 61), and *Rothowia* (BP 70) are weakly supported. No BP is given for the holophyletic *Tillandsia* and the sister group position of *Racinaea* to the clade containing *Josemania* and *Rothowia*.

Combined plastid and nuclear data (dataset D5)

Results of combined analyses of all markers used are shown in two figures. Figure 1, D5 presents a strict consensus tree summarizing mostly generic relationships. Figure 2 is more inclusive and displays relationships of all accessions investigated including subgeneric units and informal infrasubfamilial and -generic clades. These are either displayed as a phylogram from parsimony analysis with BP and PP (Figure 2, complete tree) or parts of a phylogram from Bayesian analysis showing alternative topologies (Figure 2, A–S). As in the combined nuclear dataset (Figure 1, D4), all generic entities and accepted infrasubfamilial units are holophyletic, but are here supported by mostly high or moderate values: *Glomeropitcairnia* (tribe Glomeropitcairnieae; BP 100/PP 100), *Catopsis* (tribe Pogospermeae; BP 100/PP 100), *Werauhia* (BP 100/PP 100), *Splendens* clade (BP 100/PP 100), *Chrysostachys* clade (BP 85/PP 100), *Cipuropsis* (BP 82/PP 98), *Alcantarea* (BP 100/PP 100), *Vriesea* (BP 89/PP 100), *Josemania* (BP 100/PP 100), *Racinaea* (BP 100/PP 100), Rothowia (BP 81/PP 100), and *Lemeltonia* (BP 100/PP 100); weak BP is currently given only for *Mezobromelia* (BP 66/PP 100), *Guzmania* (BP 68/PP 100), and *Tillandsia* (BP 62/PP 96). Tribes and subtribes are also strongly supported: Vrieseeae (BP 93/PP 100), Cipuropsidinae (BP 100/PP 100), Vrieseinae (BP 100/PP 100), and Tillandsieae (BP 100/PP 100). Unclassified units that were also considered in this study, but not treated taxonomically are: core Tillandsioideae (BP 100/PP 100), and core Tillandsieae (BP 93/PP 100). Relationships of the latter including genera *Josemania*, *Lemeltonia*, *Racinaea*, *Rothowia* and *Tillandsia* are resolved differently in parsimony and Bayesian analysis (Fig 2) and show currently either no BP or only PP. Infrageneric units that are well characterized and strongly to moderately supported (referring to BP only) are (1) within *Racinaea*: *R.* subg. *Pseudophytarrhiza* (BP 100/PP 100) and *R.* subg. *Racinaea* (BP 100/PP 100); (2) within *Tillandsia*: *T.* subg. *Pseudalcantarea* (BP 99/PP 100), *T.* subg. *Viridantha* (BP 95/PP 100), the *Purpurea* clade (BP 100/PP 100),

the *Gardneri* clade (BP 100/PP 100), *T.* subg. *Anoplophytum* (BP 99/PP 100), *T.* subg. *Phytarrhiza* (BP 100/PP 100), and the *Xiphioides* clade (BP 91/PP 100). Weakly supported are *T.* subg. *Pseudovriesea* (BP 68/PP 99), *T.* subg. *Tillandsia*, the *Rauhii* clade (BP 57/no PP), and the *Biflora* clade. *Tillandsia* subg. *Diaphoranthema* is paraphyletic, although internal clades receive strong statistical support (Figure 2, Q). Additional clades which are neither used formally nor named, but present in most markers studied are (1) a clade containing the *Gardneri* clade, *T.* subg. *Anoplophytum*, *T.* subg. *Diaphoranthema*, *T.* subg. *Phytarrhiza*, the *Xiphioides* clade and the unclassified species *T. albertiana*, *T. edithae*, and *T. esseriana* (BP 84/PP 100), and (2) the same but without the earlier branching *Gardneri* clade (BP 63/PP 100); and a terminal clade comprising *T.* subg. *Diaphoranthema*, *T.* subg. *Phytarrhiza*, and the *Xiphioides* clade (BP 91/PP 100).

Morphological data and analysis

Mapping morphological characters onto phylogenetic trees (data not shown) resulted in the following diagnostic traits to be used for delimiting generic clades in the present study (Table 6): ovary position, seed morphology, stigma structure, corolla structure, stamen structure, the presence vs. absence of petal appendages, and the mesophytic vs. xerophytic habit. Other characters considered to be important, but currently not used as diagnostic characters here are ovule and pollen morphology (Table 6 and 7). The main reason for not using these characters for defining genera is the high amount of missing data for many accessions, since pollen and ovules have been rarely available for detailed study. However, these characters are used to characterize new generic and subgeneric units, where data was available and most taxa could be examined. Despite of the limited sampling, the congruence between morphological characters and the molecular phylogeny is convincing. The key characters for delimiting genera in the core Tillandsioideae (all genera except *Catopsis* and *Glomeropitcairnia*) are the stigma morphology, followed by the corolla and stamen structure. Three new stigma types are recognized (conduplicate-erect, conduplicate-patent, and pinnatisect) in addition to the already described ones (Brown & Gilmartin, 1989b).

An initial trial to completely avoid the use of petal appendages as diagnostic characters failed, since some genetic entities (*Guzmania*, *Tillandsia*, internal clades of the *Cipuropsis-Mezobromelia* clade) share similar character states in their morphological traits currently used for differentiation. Therefore petal appendages are also used in the present taxonomic concept, but with a reduced taxonomic value, since no other morphological characters have yet been identified or are satisfactorily known. Petal appendages still have to be utilized for the differentiation of *Mezobromelia* from *Guzmania* and *Cipuropsis* from *Tillandsia*, with the following exceptions: (1) *T.* subg. *Pseudovriesea* and *T. malzinei* within *Tillandsia* (petal appendages present in these taxa, whereas absent from the rest of *Tillandsia* species), (2) *G. hutchisonii* within *Guzmania* (petal appendages present in this taxon, whereas absent from the rest of *Guzmania* species), (3) *W. insignis* within *Werauhia* (petal appendages absent from this taxon, whereas present in the rest of *Werauhia* species), and (4) *T. asplundii* and *T. singularis* within the *Cipuropsis-Mezobromelia* clade (petal appendages absent from these taxa, whereas present in all other *Cipuropsis-Mezobromelia* clade species). The species *Ci. amicorum* (syn. *T. amicorum*) and *M. schimperiana* (syn. *T. schimperiana*) are clearly misplaced in the genus *Tillandsia*, not only from the genetic side: all investigated material of these species exhibits petal appendages. The placement of *Ci. amicorum* into *Tillandsia* was most probably based on herbarium-preserved flowers, where petal appendages can be easily overlooked, and the

placement of *M. schimperiana* was based on a post-anthetic herbarium specimen, assuming that according to the overall morphology it would best fit into *Tillandsia*.

DISCUSSION

In contrast to "Classification system of Tillandsioideae", arrangement of discussed taxonomic units and clades is not strictly hierarchical and alphabetical and more orientated towards the tree topology (Figure 2, see Appendix). This is necessary, since additional clades that were not formally treated (e.g., core Tillandsioideae and core Tillandsieae and the *Cipuropsis-Mezobromelia* clade), and not accepted units (e.g., *T.* subg. *Allardtia*) are included as well. However, within higher units taxa and clades are arranged alphabetically to maintain clarity.

DNA data and analysis

Individual datasets D1–D3 exhibit big differences in their attributes (Table 3) and in their usefulness in resolving phylogenetic relationships, also evident from individual bootstrap consensus trees (Figure 1, D1–D3). No marker alone, neither plastid DNA (Figure 1, D1) nor individual nuclear DNA (Figure 1, D2–D3), is able to completely resolve branching patterns, but nuclear sequences in combination (on an average of about 2,300 bp) are yielding even better resolution within Tillandsioideae than combined plastid sequences (on an average of about 5,000 bp). Although the information content of nuclear datasets D2 and D3 is much higher, the amount of homoplasy also increased compared to plastid DNA data.

The easiest markers to work with so far are the *matK* gene within the plastid DNA and *PHYC* within nuclear DNA markers. *PRK* is difficult and not very suitable, especially at higher levels, in the light of the numerous indels that have to be introduced into the alignment.

Several species that were genetically investigated by multiple accessions are apparently of non-holophyletic origin (Figure 2); for some this can be explained as a result of missing DNA sequence information, evident from very short branches in respective terminal clades: e.g., *L. scaligera*, *M. schimperiana*, and *M. bicolor*; some taxa appear paraphyletic: e.g., *A. nevaresii* Leme and *R. fraseri* (Baker) M.A. Spencer & L.B. Sm.; and for others the results support a polyphyletic origin: e.g., varieties of *R. tetrantha* (Ruiz & Pav.) M.A. Spencer & L.B. Sm., *T. fendleri* Griseb., *T. guatemalensis*, some varieties and forms of *T. tectorum* E. Morren, and subspecies of *T. xiphioides*. Although this suggests the need of a critical revision of species boundaries within Tillandsioideae, sampling needs to be increased to really address this question. Therefore species circumscription is not treated within our study as it has no relevance for the outcome.

Morphological data and analysis

As already shown by previous phylogenetic and morphological studies, the four diagnostic characters used by Smith& Downs (1977) to differentiate genera within Tillandsioideae are insufficient for achieving an evolutionary based, phylogenetically accurate classification system; these morphological characters are the ovary position, apical and/or basal seed appendages, free vs. conglutinate/connate petals, and present vs. absent petal appendages. The first two characters are applied in this study in the same way for separating *Glomeropitcairnia* and *Catopsis* from core Tillandsioideae. The other two characters cannot be applied as proposed by Smith & Downs (1977), since they involve either convergent/parallel evolution or symplesiomorphic character states, evident in the evolution of a fused corolla tube in both tribes Til-

landsieae (*Guzmania*) and Vrieseeae (*Mezobromelia*) or the occurrence of petal appendages in different phylogenetic lineages. Petal appendages are primarily found in tribe Vrieseeae with only a few exceptions (see "Results"); in contrast, petal appendages are mostly absent in Tillandsieae, but also here a few exceptions are present.

Most morphological characters used in this study need a future, much detailed investigation of an increased sample of Tillandsioideae, e.g., stigma or septal nectary morphology.

Glomeropitcairnia

Glomeropitcairnia Harms. is well supported, isolated, genetically and morphologically well differentiated and comprises only two species, i.e., *Gl. erectiflora* Mez and the type species *Gl. penduliflora* (Griseb.) Mez. Till & al. (1997) illustrated and portrayed both species side by side for the first time showing important, previously undocumented morphological characteristics, i.e., a convolute-blade like stigma with unique papillae and a diffuse aperture of the pollen. The genus was originally described by Mez (1896) as a subgenus of *Pitcairnia* L'Hér. based on the character state of the position of the ½–⅔ inferior ovary. Initial controversies over the correct subfamilial placement have mostly been solved since Harms (1930) established the separate, monotypic tribe Glomeropitcairnieae under subfam. Tillandsioideae based on the common character of plumose seed appendages. Gilmartin & al. (1989) confirmed the placement within Tillandsioideae based on cladistic analyses of morphological characters and concluded the genus to be the sister taxon to *Guzmania* and *Mezobromelia*. Ranker & al. (1990) on the other hand analyzed restriction site variation of the plastid genome and suggested to treat *Glomeropitcairnia* as a monotypic subfamily within Bromeliaceae because of its genetic divergence from the other tillandsioid genera. Whereas the placement within Tillandsioideae is well supported by all published DNA sequence analyses (originally using the *ndhF* gene by Terry & Brown, 1996) a close relationship to any of the other genera and the exclusion from Tillandsioideae is not supported.

Catopsis

Other than *Glomeropitcairnia*, *Catopsis* (type species *C. nitida* (Hook.) Griseb.) was always placed within Tillandsioideae. Harms (1930) emphasized the recognition of a monotypic tribe for the genus based on its unique seed characteristics. *Catopsis* is very isolated within Tillandsioideae showing apparent pollen, septal nectary, and seed morphological characters that differentiate it from the rest of the genera within the subfamily (e.g., Böhme, 1988; Halbritter, 1992; Palací, 1997). Because of its unique position, which is also supported by a larger number of autapomorphies for the genus in the phylogenetic trees, a separate, monotypic tribe is also justified by DNA sequence data. Not Harms (1930), who established Catopsideae for *Catopsis* within Tillandsioideae as originally indicated in Barfuss & al (2005), but Brongniart (1864) was the first who proposed Pogospermeae as a monotypic tribe for *Pogospermum*, which later became a synonym of *Catopsis*. Therefore Pogospermeae is the oldest available and correct name at tribal level which has to be used.

Core Tillandsioideae = Tillandsioideae s.str.

This term for a clade found in phylogenetic investigations of Bromeliaceae was first introduced by Terry & al. (1997b) and comprised the genera *Guzmania*, *Mezobromelia*, *Tillandsia* (incl. *Racinaea*), and *Vriesea* (incl. *Werauhia*). They did not investigate any *Alcantarea* species, but Horres & al. (2000) showed that *Alcantarea* is also part of the core Tillandsioideae. Crayn & al.

(2004) had a different view in circumscribing this group and excluded *Werauhia* and *Mezobromelia*, maybe because they did not include any *Alcantarea* or true *Vriesea* species. Their only "*Vriesea*" species was *T. malzinei*, which is actually not part of the true *Vriesea* alliance (see below) but belongs to *Tillandsia* and is in sister position to *T. funckiana* Baker (Terry & al., 1997b). We are following the original idea of core Tillandsioideae and restrict the term to all currently accepted genera and informal clades except *Catopsis* and *Glomeropitcairnia*. In all phylogenetic trees obtained from different datasets (Figure 1, D1–5), regardless which genome (plastid, nuclear) was studied, core Tillandsioideae always resolve as a holophyletic unit with maximal BP.

Vrieseeae

Tribe Vrieseeae corresponds roughly to *Vriesea* s.l. (= *Vriesea* sensu Smith & Downs, 1977), with the exception of some taxa, which are now either excluded (*T. malzinei* and former xerophytic, gray-leaved *Vriesea* species) or included (*Mezobromelia* sensu typo and elements of former *T.* subg. *Allardtia*, i.e., *Ci. amicorum*, *M. schimperiana*, *T. asplundii*, and *T. singularis*). *Mezobromelia* sensu typo was only excluded in Barfuss & al. (2005), because conclusions were based on a misidentified *Guzmania* species. In the current study the tribe is subdivided into two main lineages, i.e., subtribe Cipuropsidinae with *Cipuropsis*, *Mezobromelia*, *Werauhia* and three informal clades (see below), and subtribe Vrieseinae with *Alcantarea* and *Vriesea*. Both subtribes can be distinguished by morphological characters of stigmas and ovules (see below). Vrieseeae is holophyletic in the plastid DNA tree (Figure 1, D1, BP 100), but not in the case of individual and combined nuclear markers (Figure 1, D2–D4). Although topologies of these are conflicting, supports for these alternative branching patterns involving taxa of Vrieseeae are generally weak. The reason seems to be the lack of characters to resolve backbone relationships in this part of the tree with better confidence. We still trust in the resolution of the plastid DNA tree and are fairly confident that more nuclear DNA sequences will provide the same result as plastid DNA data, since BP of the alternative topology from combined nuclear data (Figure 1, D4) still remains weak (BP 60).

Cipuropsidinae

Subtribe Cipuropsidinae is mainly composed of the Andean, Caribbean, and Mesoamerican species of *Mezobromelia* and *Vriesea* sensu Smith & Downs (1977), which do not show Vrieseinae-type stigmas. In contrast to Vrieseinae, which usually have very long appendages on the ovules, taxa of this clade generally display no or only very short appendages, except for *Vriesea tuerckheimii*. The subtribal name is based on the oldest generic name available for a species falling within this clade (*Ci. subandina*). It is divided into two main lineages, one corresponding to taxa assigned to the *Cipuropsis-Mezobromelia* clade, the other one to *Werauhia*.

Cipuropsis-Mezobromelia clade

Presently, the *Cipuropsis-Mezobromelia* clade is an assemblage of previously recognized species of *Mezobromelia* sensu typo, *Tillandsia*, and *Vriesea* (incl. *Cipuropsis*). Besides *Tillandsia* s.str, this is the second clade where a fully resolved classification system cannot yet be applied. Species and clades found in this phylogenetic unit are (1) *V. glutinosa* and *V. splendens* (*Splendens* clade), (2) *V. chrysostachys* and *V. ospinae* (*Chrysostachys* clade), (3) *V. tuerckheimii*, (4) *T. asplundii* and *T. singularis* (*Singularis* clade), (5) *Ci. amicorum* and *Ci. zamorensis* (syn. *V. zamorensis*) (*Cipuropsis*), (6) *M. bicolor*, *M. pleiosticha*, and *M. schimperiana* (*Mezobromelia*). *Vriesea* species found here differ from *Vriesea* s.str. in not having the convolute-blade or the tubolaciniate stigma morphology but either simple-erect or conduplicate-spiral, respectively. *Cipuropsis amicorum* and *M. schimperiana* have been misplaced in *Tillandsia* (petal appendages present in both species), and the *Singularis* clade lacks petal appendages and was therefore previously treated within *T.* subg. *Allardtia*, but differs clearly from that subgenus in other morphological features, e.g., pollen with fine insulae on the aperture, leaf sheaths drying silvery-grey, and tubular yellow corolla. *V. tuerckheimii* remains unclassified and without assignment to a group. We are presently proposing generic rearrangements only for three species within the *Cipuropsis* and *Mezobromelia* clades (*Ci. amicorum*, *Ci. zamorensis*, and *M. schimperiana*) mainly because of limited access to plant material for additional species that are supposed to group within the *Cipuropsis-Mezobromelia* clade. Although investigated species of this clade form distinct, moderately or strongly-supported phylogenetic units, the application of morphological characters is currently too vague to propose new genera.

Cipuropsis

Cipuropsis was proposed as a monotypic genus for *Ci. subandina* (syn. *V. subandina*). Its generic status was later accepted by Harms (1930), but not by Mez (1934–35) and Smith & Downs (1977), who treated the genus as a synonym of *Vriesea*. The only diagnostic character believed to be natural and discriminative for species of *Tillandsia* and *Vriesea* by the latter two have been petal appendages while other morphological differences between species complexes were considered to be within the morphological range of these two genera. Unfortunately, it has not been possible for us to collect or receive fresh or well-preserved plant material for genetic analyses of *Ci. subandina*, but because of its strong morphological similarity with and obvious relationships to *Ci. zamorensis* based on herbarium specimens (incl. type material) of both species (W. Till & M.H.J. Barfuss, unpubl. data), it is most likely that *Ci. subandina* species groups within this clade. As *Cipuropsis* is the oldest generic name available for a group of species within the *Cipuropsis-Mezobromelia* clade, it is restored from the synonymy of *Vriesea* and treated as a separate genus within subtribe Cipuropsidinae. Two species previously treated either under *Tillandsia* or *Vriesea* are transferred here, i.e., *Ci. amicorum* and *Ci. zamorensis*, respectively. Further taxa not investigated but supposed to belong to *Cipuropsis* are *V. dubia* (L.B Sm.) L.B. Sm., *V. duidae* (L.B Sm.) Gouda, *V. elata* (Baker) L.B. Sm., *V. lutheriana* J.R. Grant, and *V. rubra* (Ruiz & Pav.) Beer. This taxonomic change is justified, since *Ci. subandina* will always be part of the *Cipuropsis-Mezobromelia* clade. Even if the taxonomic circumscription of *Cipuropsis* will change, other species of the *Cipuropsis-Mezobromelia* clade would have to be assigned to *Cipuropsis* for reasons of priority.

Mezobromelia

Species previously classified within *Mezobromelia* s.l. belong to two phylogenetically unrelated units. The first lineage, of which we have only investigated *G. hutchisonii*, is placed within *Guzmania*; the second one, in which the type species *M. bicolor* is located (= *Mezobromelia* s.str.), is nested within the *Cipuropsis-Mezobromelia* clade as sister to *Cipuropsis*. *Mezobromelia schimperiana* is genetically definitely not a *Tillandsia* species, as evident also from flower morphology, because petals are connate and appendaged in all investigated specimens. It shares great morphological similarity with species assigned to *Mezobromelia* s.str. (J.M. Manzanares, unpubl. data), which is also evident genetically; the species is therefore assigned to this genus.

Chrysostachys clade

This clade is composed of two *Vriesea* species (the name-giving *V. chrysostachys,* and *V. ospinae*) which were already considered to be a distinct species complex under *Vriesea* (W. Till, unpubl. data). Both species show simple-erect stigma morphology.

Singularis clade

Tillandsia asplundii and the name-giving *T. singularis* are always nested within the *Cipuropsis-Mezobromelia* clade, despite having no petal appendages. Both species are sister taxa and together they are in sister position to the clade containing both genera *Cipuropsis* and *Mezobromelia*. Some similar *Tillandsia* species like *T. delicatula* L.B. Sm., *T. pinnata* Mez & Sodiro, and *T. truncata* L.B. Sm. might also cluster with these two species, but due to the lack of material, these species were not investigated genetically. This species complex was previously treated under *T.* subg. *Allardtia* and forms a distinct clade within *Cipuropsis-Mezobromelia*. Generic status might be justified, when a broader sampling has been studied.

Splendens clade

Presently it is composed of two species in northern South America. While *V. glutinosa* had been placed in *V.* sect. *Xiphion* by Smith & Downs (1977), *V. splendens* (the name-giving taxon) was classified as a member of *V.* sect *Vriesea*. The recognition of a formal status of this group, to which also *V. soderstromii* L.B. Sm. may belong, remains to be studied further.

Werauhia

Werauhia (type species *W. gladioliflora* (H. Wendl.) J.R. Grant) is holophyletic and well supported by all DNA regions studied. It is also supported by morphological characters (e.g., cupulate stigma type) that were already recognized by J.R. Grant before molecular data was available. He therefore correctly excluded these taxa from *Vriesea* and erected the genus *Werauhia* with two sections. *Werauhia* sect. *Jutleya* (type species *W. pedicellata* (Mez & Wercklé) J.R. Grant) is holophyletic, but in association with taxa of *W.* sect. *Werauhia* in two grades, making the latter paraphyletic. To retain the current classification, *W.* sect. *Werauhia* would have to be split into two groups based on the current taxon sampling. However, such actions awaits significantly increased species numbers in future studies.

Vrieseinae

Subtribe Vrieseinae is composed of the two well supported genera *Alcantarea* and *Vriesea* as they are defined in this study. Characteristics for this subtribe are ovules with usually long appendages and geographic distributions with centers of diversity in eastern Brazil. Stigma types found in this clade are convolute-blade (Brown & Gilmartin, 1989b), tubolaciniate (Leme & Brown, 2004), conduplicate-patent (Versieux & Wanderley, 2007), conduplicate-erect.

Alcantarea

Contrary to *V.* subg. *Vriesea*, *V.* subg. *Alcantarea* (Mez, 1891–94) sensu Smith & Downs (1977) (type species *A. regina* (Vell.) Harms, lectotypified by Grant & Zijlstra, 1998) is a holophyletic group if a few misplaced species are excluded (Grant, 1995a). J.R. Grant reestablished the generic status proposed by Harms (1929) based on additional morphological evidence (Grant, 1995a). Morphological characters of this genus have been recently revised by Leme (2007, 2009) and the whole *Alcantarea* by Versieux (2009) and Versieux & al. (2010, 2012). Brown & Gilmartin (1989b) and Grant (1995a) originally indicated the stigma type of *Alcantarea* to be the same as *Vriesea* (convolute-blade). Figure 23 of Brown & Gilmartin (1989b) is wrongly assigned to *V. geniculata* (Wawra) Wawra (= *A. geniculata* (Wawra) J.R. Grant) and displays a true *Vriesea* species; indications of a convolute-blade stigma morphology for other *Alcantarea* species (as *Vriesea*) in their appendix are also incorrect. Leme (2007) demonstrated that the stigma of *Alcantarea* is of a different type than that of *Vriesea*. In his opinion, the stigma is conduplicate-spiral, but this view is largely not in concordance with the original idea of this stigma type by Brown & Gilmartin (1989b). Therefore two new stigma terms are used (conduplicate-patent and conduplicate-erect, respectively) as additional morphological characters to define *Alcantarea*. These new stigma types are illustrated either in Versieux & Wanderley (2007) and Leme (2007), or Leme (2009).

Vriesea

Vriesea subg. *Vriesea* sensu Smith & Downs (1977; type species *V. psittacina*) is an assemblage of different phylogenetic units including *Werauhia*, species now assigned to the *Cipuropsis-Mezobromelia* clade, the former xerophytic, gray-leaved *Vriesea* species and *T. malzinei*, the latter two in fact belonging to *Tillandsia* (Terry & al., 1997b). Utley (1978, 1983) and Grant (1995) already recognized that *V.* subg. *Vriesea* was an assemblage of different evolutionary units, and Grant (1995) finally separated *Werauhia* (including the type species) from the rest of *V.* subg. *Vriesea* sect. *Xiphion*. Former xerophytic, gray-leaved *Vriesea* species were excluded in a series of four publications (Grant, 1993b, 1994b, 1995b, 2005). First evidence that *T. malzinei* has to be assigned to *Tillandsia* s.str. was provided by Terry & al. (1997b) and this is confirmed in the present study. Former *Vriesea* species of the *Cipuropsis-Mezobromelia* clade have never been excluded from *V.* subg. *Vriesea*, but differ clearly in not having the convolute-blade or tubolaciniate stigma type. These two stigma types are therefore the main characteristics of the predominantly Brazilian *Vriesea* species and are used to differentiate *Vriesea* s.str. in conjunction with habit or petal appendages from the remaining tillandsioid genera. Sections *Vriesea* and *Xiphion* p.p., typo excluso of *Vriesea* s.str. are para- and/or polyphyletic, but placement of species assigned to different sections is not by random in the phylogenetic context. *Xiphion* characters (included stamens) seem to be plesiomorphic within *Vriesea*, evident from clades in a grade at the base of *Vriesea*. *Vriesea* sect. *Vriesea* is derived (exserted stamens), but pa-

raphyletic, because one lineage within this clade shows *Xiphion* characters, a possible reversal to the ancestral state. This hypothesis is supported by both the parsimony and Bayesian trees, but has no statistical support, which demonstrates the need of further molecular investigations of *Vriesea* for achieving a proper infrageneric classification.

Tillandsieae

Contrary to Vrieseeae, which is currently resolved as a holophyletic unit only by plastid DNA data, Tillandsieae shows a holophyletic origin with moderate or high BP in nearly all DNA markers studied except in the *PRK* tree, where core Tillandsioideae clades display a polytomy (Figure 1, D3). This tribe contains *Guzmania*, as well as the core Tillandsieae genera *Josemania*, *Lemeltonia*, *Racinaea*, *Rothowia*, and *Tillandsia*. Since inclusion of *Mezobromelia* in Barfuss & al. (2005) was based on a misidentified *Guzmania* species and the actual type of *Mezobromelia* is placed in Vrieseeae, it is excluded here from Tillandsieae. The clade corresponds to what Crayn & al. (2004) considered as the core Tillandsioideae in their study (see above).

Guzmania

Circumscription of *Guzmania* (type species *G. monostachia*) sensu Smith & Downs (1977) has not changed over more than three decades. Some taxa were transferred from other genera to *Guzmania* after publishing of the monograph only because complete floral characteristics were available for some species and clearly indicated that they were misplaced according to the generic definition (e.g., Utley, 1978; Luther, 1998). Current DNA results mostly confirm the old circumscription with the exception of the inclusion of one morphologically distinct group of former *Mezobromelia* with laxly-branched inflorescences and exposed stamens. Out of this group only *G. hutchisonii* was investigated. This taxon was initially treated as *Tillandsia* species by Smith & Downs (1977), but was transferred to *Mezobromelia* based on the floral character of conglutinate/connate petals bearing appendages (Weber & Smith, 1983). It differs from *Mezobromelia* sensu typo (*M. bicolor*) in the inflorescence architecture (candelabra-like with compact lateral branches in *Mezobromelia* s.str. vs. laxly-branched in *G. hutchisonii*) and in the stamen structure (conglutinate anthers and included stamens in *Mezobromelia* s.str. vs. free anthers and exserted stamens in *G. hutchisonii*). Therefore, the exclusion from *Mezobromelia* s.str. and the transfer of *G. hutchisonii* to *Guzmania* is justified genetically and morphologically. In addition, evidence from plastid DNA data (M.H.J. Barfuss, unpubl. data) suggests close relationships to *G. diffusa* L.B. Sm. and *G. bakeri* (Wittmack) Mez. A number of *Guzmania* species which have not been analyzed as well (e.g., *G. candelabrum* (André) André ex Mez, *G. dalstroemii* H. Luther, *G. hirtzii* H. Luther, and *G. lemeana* Manzan.) are showing similar inflorescences architecture and therefore might also be related to *G. hutchisonii* (Smith & Downs, 1977). *Mezobromelia fulgens* L.B. Sm. is also closely related genetically (M.H.J. Barfuss, unpubl. data). Further *Mezobromelia* species with free and exposed anthers, which are supposed to be closely related to *G. hutchisonii* and therefore believed to belong to the same clade but have not been investigated, are *M. brownii* H. Luther and *M. lyman-smithii* Rauh & Barthlott. Apart from these hypotheses concerning relationships, however, a distinction between *Mezobromelia* and *Guzmania* based just on the character of petal appendages is clearly inappropriate and does not reflect true phylogenetic relationships. Whether the way how the corolla tube is developed (either early or late sympetalous; Leins & Erbar, 2010) could serve as a discriminative character (most probably truly connate in *Mezobromelia* vs. only conglutinate in *Guzmania*) needs a further, detailed investigation.

A holophyletic origin of *Guzmania* is supported by three datasets (*PHYC* D2, nuclear combined D4, and total combined D5), whereas in dataset D3 (*PRK*) *Guzmania* clades display a polytomy with other core Tillandsioideae clades (Figure 1). Present in all phylogenetic trees are three main clades of *Guzmania* taxa (shown only in Figure 2), i.e., (1) *G. hutchisonii*, (2) *Guzmania* s.str., and (3) the remaining *Guzmania* species (incl. *Sodiroa*). *Guzmania* appears paraphyletic in the plastid DNA study of Barfuss & al. (2005), but the sample investigated as *M. pleiosticha* (Griseb.) Utley & H. Luther (acc. no. MB-15 = B0015) turned out to be a misidentified *G. variegata*. *Guzmania hutchisonii* appeared as the earliest diverging lineage within Tillandsieae in Barfuss & al. (2005). This is most probably due to numerous autapomorphies for the species responsible for LBA, which is further enforced by the low number of parsimony-informative plastid DNA characters and missing samples. The genetic divergence of clades 1 and 3 from *Guzmania* s.str. (clade 2) in all markers investigated is also reflected by morphology and indicates derived status within the genus. The hypothesis that in clade 3 a recent radiation has taken place is supported by short branches relative to taxa of *Guzmania* s.str. and *G. hutchisonii*. Nevertheless, *PHYC* and combined nuclear DNA sequences data consistently place all three clades into one linage (BP 85 vs. 61, respectively) and total-combined data give good resolution, and again increasing statistical support (BP 68/PP 100) indicating the whole group to be a natural entity. Support for the separation of a genus *Sodiroa* (e.g., Baker, 1889; Harms, 1930; Mez, 1934–35; not accepted by but mentioned in the key of Smith & Downs, 1977: 1284) that is defined by connate sepals is currently not provided by DNA data, and this character probably evolved convergently or in parallel, or reversals to the ancestral state (free sepals) have occurred. This is in contrast to the acceptance by Betancur & Miranda-Esquivel (1999), who reevaluated morphological characters phylogenetically without the application of molecular tools. Further generic segregations or infrageneric divisions of clades (e.g., the three main clades) need a careful evaluation of morphological characters, more relatives of *G. hutchisonii*, and more genetic evidence to obtain well resolved relationships within *Guzmania*.

Core Tillandsieae = Tillandsieae s.str.

This clade contains all segregates of *Tillandsia* since the treatment of Smith & Downs (1977) and can be referred to *Tillandsia* s.l. (= *Tillandsia* sensu Smith & Downs, 1977) if wrongly assigned taxa are excluded or included (see below). The nested placement of the here segregated genera *Josemania*, *Lemeltonia*, *Racinaea*, and *Rothowia* within *Tillandsia* in the earlier study of Barfuss & al. (2005) obviously was an artifact caused by the low number of parsimony-informative characters. The current study clearly shows that all four segregates constitute holophyletic lineages not nested within *Tillandsia* s.str. Since *Tillandsia* sensu s.l. is morphologically very heterogeneous and the already earlier segregated genus *Racinaea* is widely accepted, recognition of *Josemania*, *Lemeltonia*, and *Rothowia* is necessary for these morphologically divergent holophyletic lineages.

Josemania

This new genus (type species *J. lindenii*) contains taxa previously ascribed to *T.* subg. *Phytarrhiza* based on the character of broad and conspicuous petal blades. In 1869 Regel proposed *T.* sect. *Wallisia* for the single species *T. lindenii*. E. Morren (1870) changed the status and erected the genus *Wallisia* which was not followed by bromeliad taxonomists. According to Art. 52.1 of the international code of botanical nomenclature (McNeill & al., 2006), this name is illegitimate at generic level and therefore the new name *Josemania* is proposed. All species

classified here, i.e., *J. anceps*, *J. cyanea*, *J. lindenii*, *J. pretiosa* and *J. umbellata*, are easily recognized by their inflorescence structure, characteristic flowers and the pinnatisect stigma morphology (already illustrated in Morren, 1871).

Lemeltonia

Species of this new genus (type species *L. dodsonii*) have also been part of former, polyphyletic *T.* subg. *Phytarrhiza* sensu Smith & Downs (1977) and appear to be the sister genus to *Tillandsia* s.str. according to the current generic circumscriptions. The genus seems to be somehow intermediate between *Racinaea*, *Rothowia*, *Josemania* on the one hand and *Tillandsia* s.str. on the other. Species assigned to this genus are *L. acosta-solisii*, *L. cornuta*, *L. dodsonii*, *L. monadelpha*, *L. narthecioides*, *L. scaligera*, and *L. triglochinoides*. *Lemeltonia narthecioides* has not been included into the recent phylogenetic analyses, but high support for the inclusion into this genus comes from previous (Barfuss & al.,2005) and unpublished DNA data of the first author as well as from morphology.

Racinaea

Racinaea (Grant, 1994a; type species *R. cuspidata*) constitutes a separate entity within tribe Tillandsieae, which remained questionable after investigation of plastid DNA markers (Barfuss & al., 2005). *Racinaea* subg. *Racinaea* consists of all species formerly assigned to *T.* subg. *Pseudo-Catopsis* plus *R. dyeriana* (syn. *T. dyeriana*), a species previously classified within *T.* subg. *Phytarrhiza* but showing similar morphological features except those of strongly asymmetrical sepals and inconspicuous petal blades. However, subsymmetric petals and conspicuous petal blades are also found in other *Racinaea* species (e.g., *R. elegans* (L.B. Sm.) M.A. Spencer & L.B. Sm., *R. riocreuxii* (André) M.A. Spencer & L.B. Sm.), which are without doubt part of this genus. *Racinaea* subg. *Pseudophytarrhiza* has been established for the two species *R. venusta* (type species) and *R. hamaleana*, which have been transferred here from *T.* subg. *Phytarrhiza* too. These two species are genetically and morphologically distinct from the rest of *Racinaea*, but phylogenetically always in sister position to *R.* subg. *Racinaea* with strong statistical support.

Rothowia

This is a very isolated linage composed of three described species, i.e., *Ro. laxissima*, *Ro. platyrhachis*, and the type species *Ro. wagneriana*, which previously have been treated under *T.* subg. *Phytarrhiza*. The genus is entirely mesophytic and displays well-established phytotelmata (water-impounding rosettes). The elate inflorescence rachises and the obconic stigma are distinctive.

Tillandsia

Tillandsia s.l. (type species *T. utriculata*, incl. *T.* subg. *Pseudo-Catopsis* = *Racinaea*) is a morphologically very heterogeneous, but holophyletic group with respect to few exceptions, which are now either found in Vrieseeae or have been transferred from there (see below). Its circumscription corresponds roughly to the clade indicated as core Tillandsieae (Figure 2). Subsequent to the monograph, parts of the genus were studied to enable better recognition of individual species groups. The segregation of *Racinaea* and *Viridantha* has caused dispute among bromeliad taxonomists, among whom the first genus is broadly accepted and the latter not.

Only on the base of our molecular data we can propose a reliable classification for several segregates (see above). However, *Tillandsia* s.str. remains in a provisional state.

Currently *Tillandsia* s.str. is circumscribed as the terminal supported backbone clade of our phylogenetic reconstruction (Figure 2) which includes either *T.* subg. *Pseudalcantarea* (parsimony analysis) or *T.* subg. *Pseudovriesea* (Bayesian analysis) as the most ancient lineage. Excluded are morphologically and genetically clearly distinguishable taxa (*Josemania*, *Lemeltonia*, *Racinaea*, *Rothowia*), which were previously treated as or integrated into separate subgenera (*T.* subg. *Pseudo-Catopsis* and meso-/semi-mesophytic *T.* subg. *Phytarrhiza*, respectively). The genus is divided into several subgenera and informal infrageneric clades, with their formal status depending mainly on phylogenetic support values (BP and PP), the number of investigated species, morphological characters to define them, and already well-established taxa in literature. As indicated under individual subgenera or infrageneric clades, their species circumscriptions have sometimes significantly changed since the monograph of Smith & Downs (1977).

Tillandsia subg. Allardtia

Tillandsia subg. *Allardtia* is not accepted under *Tillandsia*. The subgenus in the sense of Smith & Downs (1977) is an artificial assemblage of at least 14 phylogenetic units according to the current species sampling: (1) the *Singularis* clade is placed within Cipuropsidinae, (2) *T. guatemalensis* (type species of *T.* subg. *Allardtia*), *T. selleana* Harms, and *T. leiboldiana* Schltdl. are forming one group and are nested within *T.* subg. *Tillandsia* together with (3) *T. adpressiflora* Mez, (4) *T. secunda* Knuth, and (5) *T. remota* Wittm. in different positions; (6) *T. barthlottii* and *T. myriantha* are grouping with *T.* subg. *Pseudovriesea*; (7) the *Tillandsia tectorum* complex and the *Tillandsia plumosa* complex (= *Viridantha*) are closely related and form a distinct unit (*T.* subg. *Viridantha*); (8) xerophytic Andean members of *T.* subg. *Allardtia* are located in the *Xiphioides* clade together with Andean species of former *T.* subg. *Anoplophytum*; (9) *T. disticha* (i.s. = incertae sedis) is a taxon genetically very isolated from all other *Tillandsia* species; (10) *T. edithae* (i.s.) and (11) *T. australis* (i.s.) are currently unclassified, but show affinities to *T.* subg. *Anoplophytum* s.str; (12) *T. pseudomicans* (i.s.) and (13) *T. sphaerocephala* (i.s.) are also unclassified because of unstable positions in different types of analysis; (14) and the rest of *T.* subg. *Allardtia* sensu Smith & Downs (1977) containing most species seems to be a distinct holophyletic group, but presently not well supported because of low variability in both plastid and nuclear DNA markers.

Therefore the *Singularis* clade (phylogenetic unit 1) is shifted to Cipuropsidinae; *T.* subg. *Allardtia* sensu typo has to be considered as a synonym of *T.* subg. *Tillandsia* with the six taxa mentioned earlier transferred there (2–5); *T. barthlottii* and *T. myriantha* are classified within *T.* subg. *Pseudovriesea* (6); the *Tillandsia tectorum* complex is transferred to *T.* subg. *Viridantha* (7); xerophytic Andean members are transferred to the *Xiphioides* clade (8); *T. australis*, *T. disticha*, *T. edithae*, *T. pseudomicans*, and *T. sphaerocephala* are unclassified (9–13); and the rest of *T.* subg. *Allardtia* (14) is provisionally treated as a distinct clade without formal nomenclatoric adjustments (*Biflora* clade).

Tillandsia subg. Anoplophytum

Current and previous results show that *T.* subg. *Anoplophytum* sensu Smith & Downs (1977; type species *T. stricta*) is (1) paraphyletic (regarding the clade that is most inclusive for species previously classified under *T.* subg. *Anoplophytum*; see Figure 2, S (BP 84/PP 100), including

Gardneri clade, excluding *T. australis*), since (a) both *T.* subg. *Diaphoranthema* and *T.* subg. *Phytarrhiza* s.str. are nested within this clade, (b) the *Xiphioides* clade is in terminal position containing also species formerly assigned to *T.* subg. *Allardtia*, and (c) the currently unclassified *T. edithae* (*T.* subg. *Allardtia* sensu Smith & Downs, 1977) is also included; and (2) polyphyletic because *T. macbrideana* and *T. pseudomacbrideana*, elements of former *T.* subg. *Anoplophytum*, are now part of the *Biflora* clade. A postulated holophyletic origin of *T.* subg. *Anoplophytum* by Tardivo (2002) based on a phylogenetic analysis of selected morphological characters is not supported, mainly because subgeneric circumscription was according to Smith & Downs' vague morphological definition. The distinction between *T.* subg. *Anoplophytum* and *T.* subg. *Allardtia* based on the character of "plicate vs. straight" filaments is obviously inappropriate and does not reflect true relationships. Support for this view comes not only from the phylogenetic results but also from ontogenetic studies on species from other subgenera (Evans & Brown, 1989), where plicate filaments are also reported for *T.* subg. *Allardtia* and *T.* subg. *Tillandsia*. In its current circumscription *T.* subg. *Anoplophytum* is restricted to *T. stricta* and its close relatives, which have their distribution center in south-eastern Brazil. The taxon is characterized by simple inflorescences with polystichously arranged flowers.

Tillandsia subg. Diaphoranthema

In the current analyses *T.* subg. *Diaphoranthema* (type species *T. recurvata* (L.) L.) is a paraphyletic lineage within *Tillandsia*, but this is not well supported. The investigated species are forming a clade in the parsimony analysis in sister position to the *Xiphioides* clade, but with *T.* subg. *Phytarrhiza* s.str. nested inside. In the Bayesian analysis the three clades composed of species of *T.* subg. *Diaphoranthema* (Figure 2, S), i.e., *Capillaris* clade (*T. capillaris*, *T. funebris* A. Cast., *T. kuehasii* W. Till, and *T. virescens*) *Usneoides* clade (*T. landbeckii* ssp. *andina* W. Till, *T. mollis* H. Hrom. & W. Till, *T. usneoides* (L.) L., and *T.* cf. *usneoides*), and *T. recurvata*, are associated in different degrees with both *T.* subg. *Phytarrhiza* and the *Xiphioides* clade. To test for a holophyletic origin of *T.* subg. *Diaphoranthema* in the current circumscription additional DNA markers and taxa are required. The stigma type of all species in this subgenus is simple-erect according to Brown & Gilmartin (1989b), lobes are short rendering a capitate stigma resembling that of *T.* subg. *Phytarrhiza* s.str. This morphological similarity points towards a close relationship of both subgenera in their actual circumscription (Till, 1992). Both taxa share an orange pollen color which is absent in remaining *Tillandsia*.

Tillandsia subg. Phytarrhiza

Tillandsia subg. *Phytarrhiza* sensu Smith & Downs (1977; type species *T. duratii*) is clearly artificial and polyphyletic. The division into mesophytic, semi-mesophytic and xerophytic members was a first attempt of grouping these species into natural units (Gilmartin, 1983; Gilmartin & Brown, 1986; Till, 2000b). This view is only partly supported by DNA sequence data. Mesophytic and semi-mesophytic members belong to four different evolutionary units, three of them representing separate phylogenetic entities, which deserve generic status (*Josemania*, *Rothowia*, *Lemeltonia*). The fourth, containing *R. dyeriana*, *R. hamaleana* and *R. venusta*, is strongly associated with *Racinaea* and therefore these species are transferred to this genus. Xerophytic members likewise are a polyphyletic group within *Tillandsia*. They also display at least two phylogenetically distinct units, (1) species associated with *T. purpurea* Ruiz & Pav. (*Purpurea* clade), and (2) species around *T. duratii*, showing strong affinities to *T.* subg. *Diaphoranthema*. In the current treatment *T.* subg. *Phytarrhiza* is restricted to *T. duratii* and its close

relatives and is accepted as a separate subgenus, despite of its nested position (see "Classification of Tillandsioideae" for reasons). Other taxa examined so far are *T. kirschnekii* Rauh & W. Till, *T. paleacea* C. Presl, and *T.* aff. *streptocarpa* Baker. Whether its subgeneric status can be kept or *T.* subg. *Diaphoranthema* must be merged as suggested by Till (1992) needs further investigation of more species and additional DNA markers to obtain well-resolved relationships.

Polyphyletic *Tillandsia* subg. *Phytarrhiza* in the sense of Smith & Downs (1977) has been studied by Gilmartin (1983) and Gilmartin & Brown (1986) in more detail, and by Till (1992) for a morphological comparison to *T.* subg. *Diaphoranthema* (see above). The first two studies used cladistic analyses of morphological characters (mainly obtained from Smith & Downs, 1977) primarily to test hypotheses concerning evolution of xerophytes and mesophytes within the subgenus. The main problem of both studies is the assumption that *T.* subg. *Phytarrhiza* is holophyletic, which does not match the molecular results. Their postulation for monophyly is based on the character of broad and conspicuous petal blades, which is obviously a character that evolved convergently or in parallel in different lineages. Identification of species groups within *T.* subg. *Phytarrhiza* (and *T.* subg. *Diaphoranthema* as sister or outgroup) of Gilmartin & Brown (1986) prior to the computer analyses for phylogenetic reconstruction is very critical and resulting trees should be taken with caution. Initial analyses of Gilmartin (1983) are more interesting, since analyses are based on single species and not on species groups. Three important clades from her phenetic analysis correspond to the newly erected genera *Josemania*, *Lemeltonia*, and *Rothowia*. The morphological study of *T.* subg. *Diaphoranthema* and *T.* subg. *Phytarrhiza* by Till (1992) support close relationships of both subgenera in their current circumscriptions.

Tillandsia subg. Pseudalcantarea

Tillandsia subg. *Pseudalcantarea* currently consist of three species: *T. grandis* Schltdl., *T. macropetala* , and *T. viridiflora* (type species). Further species that were previously assigned to this subgenus belong to different phylogenetic units. *T. baliophylla* lies within the *Biflora* clade and *T. paniculata* within *T.* subg. *Tillandsia*; therefore these two species are excluded from *T.* subg. *Pseudalcantarea*. *Tillandsia heterophylla* E. Morren, a simulator of morphological characters fitting the description of *T.* subg. *Pseudalcantarea* (Till, 2000b), also appears not closely related to *T.* subg. *Pseudalcantarea*. Instead, this species is part of group III species of Gardner's classification of *T.* subg. *Tillandsia* (Gardner, 1982, 1986b), which are, however, located in the *Biflora* clade and not part of *T.* subg. *Tillandsia*. The view of Beaman & Judd (1996) that *T.* subg. *Pseudalcantarea* should be restricted only to the species *T. viridiflora* and *T. baliophylla*, and the transfer of *T. grandis* to *T.* subg. *Tillandsia* is not supported by neither plastid DNA nor nuclear DNA sequence data. *Tillandsia* subg. *Pseudalcantarea* with its three here accepted species forms a distinct group being sister to the remaining clades of *Tillandsia* in the parsimony analysis (Figure 2), but this position is only weakly supported and not present in Bayesian analysis (Figure 2, R, here *T.* subg. *Pseudovriesea* is the earliest branch within *Tillandsia* s.str.). Future studies including more DNA markers will help clarifying the correct phylogenetic position of this subgenus which might merit generic status.

Tillandsia subg. Pseudovriesea

The new *T.* subg. *Pseudovriesea* (type species *T. tequendamae*) is composed of all xerophytic, grey-leaved species previously assigned to *Vriesea* and some *Tillandsia* species which were

either never formally transferred to *Vriesea*, despite having petal appendages (i.e., *T. myriantha*, formerly part of *T.* subg. *Allardtia*), or because the investigated material did not show appendages on the petals so far (*T. barthlottii*, *T. spathacea*; the former being previously part of *T.* subg. *Allardtia*, the latter of *T.* subg. *Tillandsia*). J.R. Grant already recognized the close relationship to *Tillandsia* based on overall morphological similarity (habit) and differences in stigma morphology to *Vriesea* s.str. (convolute-blade or tubolaciniate vs. conduplicate-spiral). He transferred all xerophytic, grey-leaved *Vriesea* species to *Tillandsia*, at first those with exserted stamens and styles (Grant, 1993b, 1994b, 1995b), later also species with included stamens and styles (Grant 2005). The main difference to *T.* subg. *Tillandsia* is that most of these species have retained (or regained) their petal appendages, a character which is rarely found in other species of *Tillandsia*. Unfortunately, petal appendages are not synapomorphic for all taxa within *T.* subg. *Pseudovriesea*, since few members do not show petal appendages so far in the investigated flowers. To really address this issue populational, investigations on petal appendages would be necessary.

The long standing debate on whether xerophytic, grey-leaved *Vriesea* species are members of *Vriesea* (Smith & Downs, 1977), of *T.* subg. *Tillandsia* (Grant, 1993b, 1994b, 1995b), or of a separate phylogenetic unit within *Tillandsia* (Barfuss & al., 2005), is now resolved. They fall neither into *Vriesea* s.str. nor into *T.* subg. *Tillandsia* (as Grant originally thought when he did the first rearrangements of taxa with exserted stamens and styles), but they are clearly members of *Tillandsia* deserving a subgenus of their own. Evidence from all plastid and nuclear DNA markers are supporting this view and earlier speculations of a possible ancient hybrid origin of this group between any *Vriesea* and *Tillandsia* species can be rejected.

Tillandsia subg. Tillandsia

Species of *T.* subg. *Tillandsia* were consistently separated from the other subgenera since Mez (1896) based on the floral characters of exserted stamens and styles, which was followed by Smith & Downs (1977). Gardner (1982, 1986b) established 5 groups within *T.* subg. *Tillandsia* sensu Smith & Downs (1977), but also included some possibly wrongly classified *Allardtia* species. Based on results of the current phylogeny, this subgenus should be restricted (1) to group I and II species of her classification, (2) to *T. filifolia* Schltdl. & Cham. of group IV, which in her investigations shows also affinities to group II, as well as (3) to some taxa previously assigned to *T.* subg. *Allardtia* (*T. adpressiflora*, *T. guatemalensis*, *T. leiboldiana*, *T. remota*, *T. secunda*, *T. selleana*). As the type species of *T.* subg. *Allardtia*, *T. guatemalensis*, is nested within *T.* subg. *Tillandsia*, *Allardtia* necessarily becomes a synonym of *Tillandsia* subg. *Tillandsia*. Group V species are actually species of *T.* subg. *Viridantha*, group III species are mainly nested within the *Biflora* clade and the second species of her group IV, *T. disticha*, shows currently no supported affinity to any infrageneric unit within *Tillandsia* and is therefore unclassified. Wrongly assigned is *T. rauhii* (group III, now *Rauhii* clade). Some species with undetermined affiliation in Gardner's treatment can now be assigned to clades within *Tillandsia*: *T. cryptopoda* L.B. Sm. and *T. plagiotropica* Rohweder clearly belong to *T.* subg. *Tillandsia*, group I; *T. extensa* Mez is part of group II, *T. ecarinata*, *T. ferreyrae* and *T. teres* are members of the *Rauhii* clade and not supported to belong to *T.* subg. *Tillandsia*. Support for subgroups of Gardner's group I (1986b) is currently also not given (see also Granados Mendoza, 2008).

Although the subgenus has currently no statistical support, validation for the inclusion of the investigated taxa comes from previous studies and unpublished data (Terry & al., 1997b; Barfuss & al., 2005; M.H.J. Barfuss, unpubl. data). Four main clades can be recognized: (1) a

clade containing *T. secunda* (*T.* subg. *Allardtia* sensu Smith & Downs, 1977), *T. malzinei* (*V.* sect. *Vriesea* sensu Smith & Downs, 1977), and *T. paniculata* (*T.* subg. *Pseudalcantarea* sensu Smith & Downs, 1977); all these taxa have not been included into *T.* subg. *Tillandsia* before; (2) a clade that W. Rauh considered as the *Tillandsia mima* complex (Rauh, 1973; group II species of Gardner, 1986b) including *T. adpressiflora* (*T.* subg. *Allardtia* sensu Smith & Downs, 1977) and *T. hildae* Rauh (group II, Gardner, 1986b); (3) a clade which contains *T. utriculata* and therefore could be recognized as *T.* subg. *Tillandsia* s.str. (also group II species plus *T. filifolia* of group IV, Gardner, 1986b); and (4) a clade containing the majority of species (group I species, Gardner, 1986b, plus *T.* subg. *Allardtia* s.str.). Interesting are the geographical distributions of these clades. Whereas the early diverging clades 1 and 2 are outside the diversity center of *T.* subg. *Tillandsia* and mainly found in northern South America and the Caribbean, the two core clades have their main distribution in Mexico and Central America. This pattern gives important hints for future biogeographical studies in Tillandsioideae.

Pseudobulbous taxa of *T.* subg. *Tillandsia* were studied by Chew & al. (2010). Results based on ITS 2 and ETS data support the polyphyletic origin of this habit within *T.* subg. *Tillandsia* and even within the whole genus *Tillandsia*, although statistical support unfortunately is mostly weak. An enriched taxon sampling and additional molecular markers would help clarifying the interesting question, how often the pseudobulbous habit arose within *Tillandsia*.

Tillandsia malzinei is a special case within *T.* subg. *Tillandsia*. Genetically, from both nuclear and plastid DNA data, this species is doubtlessly nested in the *Tillandsia* clade, despite bearing appendages at the base of the petals. It is genetically very distinct from other *Tillandsia* species, but shows some genetic affinities to *T. funckiana* (Terry & al., 1997b) and other members of *T.* subg. *Tillandsia*, but not to *T.* subg. *Pseudovriesea*. Therefore we are currently treating this species as a strongly isolated taxon within *T.* subg. *Tillandsia*, due to its high number of molecular autapomorphies and the distinct morphology.

Tillandsia subg. Viridantha

Viridantha (Espejo-Serna, 2002) with *T. plumosa* as type species does not deserve generic rank under subfam. Tillandsioideae based on the current generic circumscriptions. Taxa of *Viridantha* clearly are member of *Tillandsia* s.str., but not related to species of *T.* subg. *Tillandsia* (group V: Gardner, 1982, 1986b) or associated with the *Biflora* clade (former *T.* subg. *Allardtia* sensu Smith & Downs, 1977). This is also supported by flower morphology (Brown & Gilmartin, 1989b) since some *Viridantha* species resemble the convolute-blade stigma type (W. Till & M.H.J. Barfuss, unpubl. data; but also illustrated in Gardner (1982) and Espejo-Serna (2002)), a character not found in any other subgroup of *Tillandsia*. Instead, these taxa are closely related to the *Tillandsia tectorum* complex (Hromadnik, 2005), which was previously included in *T.* subg. *Allardtia* and *T.* subg. *Anoplophytum* sensu Smith & Downs (1977). We therefore consider species of *Viridantha* and the *Tillandsia tectorum* complex as members of a separate subgenus. An internal classification of this subgenus needs a deeper and more comprehensive study of all species assigned to *T.* subg. *Viridantha*. The sectional treatment of Espejo-Serna (2002: *Vi.* sect. *Viridantha* and *Vi.* sect. *Caulescens*) is currently not supported. Descriptions of new taxa during the last few years and monographic treatments of both species complexes (e.g., Espejo-Serna, 2002; Hromadnik, 2005; Ehlers, 2009) using traditional, morphology based taxonomy show the need of a taxonomic revision also on a (phylo-)genetic basis.

The placement of *T. sphaerocephala* as sister to *T.* subg. *Viridantha* in the parsimony analysis seems to be an artifact with no statistical support caused by low sequence variability and

the lack of other species related to this taxon. Bayesian analysis places *T. sphaerocephala* in sister position to the *Purpurea* clade (Figure 2, L), which in the parsimony analysis groups with unclassified *T. nana* and *T. pseudomicans*, a possible artifact as well (PP 60).

Biflora clade

This is one of the most critical species complexes within *Tillandsia* (name-giving taxon *T. biflora*) and comprises mostly taxa previously assigned to *T.* subg. *Allardtia*. Some members of this clade have also been classified in other subgenera, but clearly belong into this phylogenetic unit: (1) most group III species of Gardner's treatment of *T.* subg. *Tillandsia* (Gardner 1982, 1986b), i.e., *T. deppeana*; *T. heterophylla*, *T. imperialis*, and *T. multicaulis*, (2) *T. baliophylla* of *T.* subg. *Pseudalcantarea* sensu Smith & Downs (1977), and (3) elements of *T.* subg. *Anoplophytum* sensu Smith & Downs (1977), i.e., *T. macbrideana* and *T. pseudomacbrideana*. Because the type species of *T.* subg. *Allardtia*, *T. guatemalensis*, is now part of *T.* subg. *Tillandsia*, a new subgenus would have to be created for these taxa. DNA data weakly links the species to a holophyletic group with no statistical support and definite morphological characters are currently missing. Therefore, we keep this complex as an informal clade under *Tillandsia*. Although the huge majority of species was previously classified within *T.* subg. *Allardtia*, included style and stamens with no filament plication can obviously not serve as an autapomorphic character for this complex.

Gardneri clade

Earlier, this group of species (name-giving taxon *T. gardneri*) was classified within *T.* subg. *Anoplophytum*. It is composed only of rose-flowered species distributed in eastern Brazil. The DNA results suggest that this species complex has had a relatively long time of independent evolution. Ehlers (1997) monographed them as "red-flowered" species and included 14 Brazilian members plus *T. paraensis* Mez. The latter belongs to *T.* subg. *Tillandsia* but was not investigated. We investigated only 3 species (*T. brachyphylla*, *T. gardneri*, *T. globosa*) and are not confident enough to propose a separate formal unit for these taxa. Therefore, this complex is recognized as an informal clade within *Tillandsia*.

Purpurea clade

As a part of former *T.* subg. *Phytarrhiza*, this clade contains xerophytic members (name-giving taxon *T. purpurea*) and is in an isolated position within *Tillandsia* and not closely related to any other subgroup. It comprises the following taxa investigated so far: *T. aurea*, *T. cacticola*, *T. purpurea*, and *T. straminea*. Species of the *Purpurea* clade are distinguished from *T.* subg. *Phytarrhiza* s.str. (simple-erect stigma type) by its conduplicate-spiral stigmas (Brown & Gilmartin, 1989b) and the often bicolored petals (crème-colored with violet tips), but for establishing a separate subgenus more taxa and accessions have to be investigated.

Tillandsia nana and *T. pseudomicans* are sister to this clade in the parsimony analysis, but with weak BP (57). In Bayesian analysis *T. sphaerocephala* is sister to the *Purpurea* clade, but also only weakly supported. Both scenarios are probably an artifact caused by missing *Tillandsia* taxa and sequence information.

Rauhii clade

Species of the *Rauhii* clade (*T. ecarinata*, *T. ferreyrae*, the name-giving *T. rauhii*, and *T. teres*) have been previously classified within *T.* subg. *Tillandsia* (Smith & Downs, 1977). Gardner

(1982, 1986b) treated *T. rauhii* within group III of *T.* subg. *Tillandsia*, but this taxon is neither related to this group of species, nor currently placed within *T.* subg. *Tillandsia*. *Tillandsia ecarinata*, *T. ferreyrae* and *T. teres* were not classified into any subunit of *T.* subg. *Tillandsia* and treated as "incertae sedis" by Gardner (1982, 1986b), since floral characteristics could not be studied at that time. Despite of missing plastid DNA sequence data for all taxa except *T. rauhii*, placement of all four species within the *Rauhii* clade is still supported in parsimony analyses with a weak BP (57). The *Rauhii* clade shows an isolated position within *Tillandsia*, but backbone relationships between this and other phylogenetic units are unsupported. Placement is very likely to change if more DNA sequence data is gathered. Thus no formal unit is proposed for species of the *Rauhii* clade until placement of these taxa is clarified.

Xiphioides clade

Taxa falling into this phylogenetic unit (name-giving taxon *T. xiphioides*) have been mainly placed within *T.* subg. *Anoplophytum* based on the character of plicate filaments. According to the tree topologies, this group constantly is closely related to subgenera *Anoplophytum* s.str., *Diaphoranthema*, *Phytarrhiza* s.str., the *Gardneri* clade and some unclassified taxa, i.e., *T. albertiana*, *T. edithae* and *T. esseriana* (Figure 2). Till (2000b) recognized close relationships of taxa under both *T.* subg. *Allardtia* and *T.* subg. *Anoplophytum* sensu Smith & Downs (1977), but shifted the xerophytic Andean members of *T.* subg. *Anoplophytum* with plicate filaments to *Allardtia*. Genetically they are neither related to *T.* subg. *Allardtia* s.str. (which is now a synonym of *T.* subg. *Tillandsia*) nor to the rest of former *Allardtia* (= *Biflora* clade). From an evolutionary point of view it would have been better to shift xerophytic Andean members of *T.* subg. *Allardtia* with straight filaments into *T.* subg. *Anoplophytum*. As *T.* subg. *Anoplophytum* is currently circumscribed, the *Xiphioides* clade would need a separate infrageneric unit. However, due to the unresolved relationships of *T.* subg. *Phytarrhiza* and *T.* subg. *Diaphoranthema* and the unclassified taxa mentioned earlier, as well as currently missing morphological characteristics, the *Xiphioides* clade is treated here as an informal unit within *Tillandsia*.

Incertae sedis—Unclassified Tillandsia species

Taxonomists always try to put taxa into a hierarchical scheme. Unfortunately there are a number of *Tillandsia* species that show either no or only unsupported affinities to certain groups, but appear as separate, maybe relict ancient lineages, where their ancestors either did not diversify or related species became extinct or have yet not been discovered. Unclassified investigated taxa so far are *T. albertiana*, *T. australis*, *T. disticha*, *T. edithae*, *T. esseriana*, *T. nana*, *T. pseudomicans* and *T. sphaerocephala*. Morphological characteristics of these species seem to be either plesiomorphic or derived involving convergence or parallelism. Currently we cannot assign these species with good phylogenetic support values to any infrageneric unit. Additional taxa and new molecular markers may help to place these taxa with better confidence in the phylogenetic framework.

Classification of Tillandsioideae

In classifying Tillandsioideae we considered aspects of biological (evolutionary) classification of Hörandl & Stuessy (2010) and agree in principle with statements of Nickrent & al. (2010), who gave a good overview of their philosophy in reclassifying *Santalales* into different families. Although their classification was done on a higher level, their principal points apply in our view to any taxonomic level. Classifications are most useful when they simultaneously serve many functions as stressed by both studies mentioned above. Nickrent & al. (2010) list several criteria used to decide how to circumscribe taxonomic units. For the taxonomy of Tillandsioideae we are currently emphasizing the following (Nickrent & al., 2010, slightly modified), arranged according to their importance : (1) rejection of polyphyly, support for holophyly (= monophyly s.str.) and paraphyly, if well supported, (2) phylogenetic information (= minimizing redundancy), (3) stability (= minimizing nomenclatural changes), (4) ease of identification (= recognizability), (5) recognition of groups that are well-established in the literature, but where supporting information is currently partly missing. Hörandl & Stuessy (2010: 1650) list also four main criteria (theoretical foundation based on natural processes, predictivity, information content, and practicability), which are similar to those mentioned above, and they recommend to adhere to these principles of biological classification by following a protocol of five procedural steps. They emphasized the recognition of paraphyletic groups as natural units of biological classification. Contrary to Nickrent & al. (2010) we agree with their opinion that paraphyly is a natural stage in the evolution of taxa, and that it occurs regularly along with holophyly. However, in our view it should only be accepted for a final classification if all possible biological sources are considered and well-supported conclusions can be drawn. For an acceptance of conclusive, well-supported paraphyletic groups within Tillandsioideae, information from biological sources (in this study from DNA sequence data and morphology) is currently insufficient. Statistically unsupported paraphyly is preliminarily accepted for one already well-established taxon (*T.* subg. *Diaphoranthema*), where additional information could also lead to holophyly; unsupported paraphyly may also be tentatively accepted in a not yet fully resolved classification for a group, in which additional information is currently missing; it may be most relevant for most-recently divergent plant groups and at the species level. Therefore, until conclusive and well-supported clades in the complete phylogeny of all Tillandsioideae are presented, we are not describing or accepting new taxonomic units based on paraphyly, but it could be of major importance in a future, more detailed classification than presented here. Our main attempts are to adhere to the above emphasized five principles. Units that are well characterized are formally recognized, whereas others, for which significant supporting information is missing, are treated as informal clades or unclassified taxa (= incertae sedis) in our classification system.

The subfamilial classification including accepted tribal, subtribal, generic and subgeneric ranks and informal clades is listed below in hierarchical, followed by alphabetical order. Two new subtribes (Cipuropsidinae, Vrieseinae), three new genera (*Josemania*, *Lemeltonia*, *Rothowia*), and three new subgenera (*Racinaea* subg. *Pseudophytarrhiza*, *T.* subg. *Viridantha*, and *T.* subg. *Pseudovriesea*) are described. Several species are therefore reclassified. Lectotypes are selected for *Catopsis* subg. *Tridynandra* Mez, *Tillandsia* sect. *Conostachys* Griseb., *Tillandsia* sect. *Eriophyllum* K. Koch. A comparison of accepted genera of Tillandsioideae and subgenera of *Tillandsia* to previous classification systems of either Smith & Downs (1977), Smith & Till (1998), Till (2000a, b), or Barfuss & al. (2005) can be found in Tables 4 and 5, re-

spectively. Phylogenetic relationships of all investigated species and their current classification are summarized in Figure 2 (see Appendix). A complete synonymy (homotypic and heterotypic) is presented for all accepted taxa above the species level and for all reclassified species, taxonomic synonyms with the monograph of Smith & Downs (1977) being the primary reference are given with investigated species mentioned to allow a comparison of the new vs. old taxonomic circumscriptions in the most critical groups. Morphological characteristics are listed for all taxa above the subgeneric level and for newly described infrageneric units and the geographical distributions of well-characterized clades can be obtained from the key to the genera.

Bromeliaceae Juss. subfamily **Tillandsioideae** Burnett, Outl. Bot.: 442 (Jun 1835), as "Tillandsidae".—Type: *Tillandsia* L.

Fruit a septicidal capsule, seeds with hairs or hairlike appendages, wind dispersed.

Tribe **Glomeropitcairnieae** Harms, in Engl. & Prantl, Nat. Pflanzenfam. ed. 2, 15a: 100, 115 (1930).—Type: *Glomeropitcairnia* (Mez) Mez.

Ovary about ½–⅔ inferior, capsule only partly septicidal; petal appendages present; stigma of the convolute-blade type; seeds with a long filiform, undivided chalazal appendage and a plumose micropylar flight apparatus; pollen with a diffuse aperture, exine reticulate.

1. *Glomeropitcairnia* (Mez) Mez, Bull. Herb. Boissier, sér. 2, 5: 232 (1905) ≡ *Pitcairnia* subg. *Glomeropitcairnia* Mez, in C.DC., Monogr. phan 9: 463 (1896).—Type: *Tillandsia penduliflora* Griseb.

Tribe **Pogospermeae** Brongn., Ann. Sci. Nat. Bot., sér. 5, 1: 327 (1864).—Type: *Pogospermum* Brongn. (= *Catopsis* Griseb.).

= Bromeliaceae tribe Catopsideae Harms, in Engl. & Prantl, Nat. Pflanzenfam. ed. 2, 15a: 100, 130 (1930).—Type: *Catopsis* Griseb.

Ovary max. ⅛ inferior to completely superior, capsule septicidal; petal appendages lacking; stigma of the simple-erect type; flight apparatus of the seeds of multicellular chalazal hairs; pollen with sharply cut aperture margins, exine reticulate (*Catopsis* type).

2. *Catopsis* Griseb., Nachr. Königl. Ges. Wiss. Georg-August-Univ. [1]: 10, 12 (13 Jan 1864) ≡ *Catopsis* subg. *Eucatopsis* Mez, in C.DC., Monogr. phan. 9: 619 (1896).—Type: *Tillandsia nitida* Hook.

= *Tussacia* Willd. ex Beer, Fam. Bromel.: 99 (1856), non Rchb., Iconogr. bot. exot. 1: x, 28 (1827: Gesneriaceae) nec Willd. ex Schult. & Schult. f., in Roem. & Schult., Syst. veg. 7(1): x, 57 (1829: Incertae sedis), nom illeg. (Art. 53.1) ≡ *Tussaria* Griseb., Fl. Brit. W. I.: 599 (1864), orth. var.—Type: *Tillandsia vitellina* Klotzsch = *Catopsis nutans* (Sw.) Griseb.

= *Pogospermum* Brongn., Ann. Sci. Nat. Bot. Sér. 5, 1: 327 (Jun 1864).—Type: *Pogospermum flavum* Brongn. = *Catopsis nutans* (Sw.) Griseb.

= *Catopsis* subg. *Tridynandra* Mez, in C.DC., Monogr. phan. 9: 620 (1896).—Lectotype (proposed): *Catopsis morreniana* Mez.

Tribe **Tillandsieae** Rchb., Consp. regn. veg.: 62 (Dec 1828–Apr 1829) ≡ Bromeliaceae tribe Tillandsieae Dumort., Anal. Fam. Pl.: 55 (Oct 1829) ≡ Bromeliaceae tribe Tillandzieae Rchb., in Uphof, Pflanzengattungen: 205 (1910), orth. var.—Type: *Tillandsia* L.

Ovary for max. ⅓ inferior, capsule septicidal; petal appendages usually lacking; stigma mainly of the conduplicate-spiral or simple-erect type, rarely of the convolute-blade type (*Guzmania* p.p., *Rothowia*, *Tillandsia* subg. *Viridantha* p.p.), occasionally of the coralliform type (*Lemeltonia*, *Racinaea* p.p.) or with pinnatisect margins (*Josemania*); seeds with a flight apparatus of pseudohairs at the micropylar end, endostome of type d (Groß, 1988) and embryo of type g (Groß, 1988); pollen mainly with diffuse apertures, occasionally of the insulae type, rarely of the *Alcantarea* type (former *Vriesea imperialis* type; Halbritter, 1988, 1992), or inaperturate.

3. *Guzmania* Ruiz & Pav., Fl. peruv. 3: 37, pl. 261 (1802).—Type: *Guzmania tricolor* Ruiz & Pav.
= *Guzmania monostachia* (L.) Rusby ex Mez.
= *Caraguata* Plum. ex Lindl., Bot. Reg. 13: sub pl. 1068 (1827), nom. illeg. (Art. 6.4), non Plum. ex Adans. (1763).—Type: *Tillandsia lingulata* L.
= *Devillea* Bertero ex Schult. & Schult. f., in Roem. & Schult., Syst. veg. 7(2): 1229 (1830).—Type: *Devillea speciosa*, nom. nud.
= *Tillandsia* sect. *Conostachys* Griseb., Nachr. Königl. Ges. Wiss. Georg-August-Univ. [1]: 19 (13 Jan 1864) ≡ *Tillandsia* subg. *Conostachys* (Griseb.) Baker, J. Bot. 26: 167 (1888) ≡ *Vriesea* subg. *Conostachys* (Griseb.) Mez, in Mart., Eichler & Urban, Fl. bras. 3(3): 516 (1894).—Lectotype (proposed): *Tillandsia acorifolia* Griseb.
= *Massangea* E. Morren, Belgique Hort. 27: 59, 199, pl. 8, 9 (1877) ≡ *Caraguata* [Plum. ex Lindl.] subg. *Massangea* (E. Morren) Baker, Handb. Bromel.: 149 (1889).—Type: *Tillandsia musaica* Linden & André.
= *Sodiroa* André, Bull. Soc. Bot. France 24: 167 "1877" (1878).—Lectotype (Smith & Downs, 1977: 1275): *Sodiroa graminifolia* André ex Baker.
= *Schlumbergeria* E. Morren, Belgique Hort. 28: 311 (1878) ≡ *Schlumbergera* E. Morren, Belgique Hort. 33: 46 (1883), orth. var., non Lem. (1858: Cactaceae) ≡ *Caraguata* [Plum. ex Lindl.] subg. *Schlumbergeria* (E. Morren) Baker, Handb. Bromel.: 149 (1889).—Type: *Schlumbergeria roezlii* E. Morren.
= *Thecophyllum* André, Bromel. Andr.: 107 (1889).—Type: *Thecophyllum wittmackii* André.
= *Chirripoa* Suess., Bot. Jahrb. Syst. 72: 293, pl. 4, Fig. 11 (1942).—Type: *Chirripoa solitaria* Suess. = *Guzmania polycephala* Mez & Wercklé ex Mez
– *Mezobromelia* L.B. Sm. p.p., typo excluso.

Mesophytic, usually acaulescent; flowers mostly polystichously arranged; petals connate/conglutinate and mostly unappendaged; filaments free, anthers free or connate; pollen inaperturate or with diffuse aperture; stigma of the simple-erect or convolute-blade type; seeds with an endostome of type a and b, rarely e (Groß, 1988) and embryo of type a, rarely b (Groß, 1988).

Guzmania hutchisonii (L.B. Sm.) Barfuss & W. Till, **nom. prov.** ≡ *Tillandsia hutchisonii* L.B. Sm., Phytologia 13: 145, pl. 7, figs. 23, 24 (1966) ≡ *Mezobromelia hutchisonii* (L.B. Sm.) W. Weber & L.B. Sm., J. Bromeliad Soc. 33(3): 121 (1983).—Type: Hutchison & Wright 6801: US (holo), UC, USC (iso).

= *Mezobromelia trollii* Rauh, Trop. Subtrop. Pflanzenwelt 21: 5, figs. 1–3 (1977).—Type: Rauh 40104: HEID (holo), US (iso).

4. *Josemania* Barfuss & W. Till, **nom. prov.** pro *Wallisia* (Regel) E. Morren ≡ *Tillandsia* sect. *Wallisia* Regel, Index Seminum Hort. Bot. Petrop. "1868": 92 (Mar 1869) ≡ *Wallisia* (Regel) E. Morren, Belgique Hort. 20: 97 (1870), nom. illeg. (Art. 52.1) ≡ *Vallesia* C. Wright ex Sauvalle, Anales Acad. Ci. Méd. Fís. Nat. Habana 8: 53 (1871), orth. var., non *Vallesia* Ruiz & Pav. (1794: Apocynaceae) ≡ *Tillandsia* subg. *Wallisia* (Regel) Baker, J. Bot. 26: 46 (1888).—Type: *Tillandsia lindenii* Regel.

– *Tillandsia* subg. *Phytarrhiza* (Vis.) Baker p.p., typo excluso.

Leaves narrowly triangular, usually longitudinally reddish striped near the base, inflorescence distichous, usually ovate-laceolate in outline (except *Josemania umbellata*), petals blue-violet with a strongly widened blade and constricted basally into a claw, stamens and style deeply included within the corolla, anthers subbasifixed, stigma lobes with pinnatisect and papillate margins, ovules slenderly cylindric, obtuse.

Named after José Manuel Manzanares (1957–) from Quito, Ecuador, leading authority of Ecuadorian Bromeliaceae.

Josemania anceps (Lodd.) Barfuss & W. Till, **nom. prov.** ≡ *Tillandsia anceps* Lodd., Bot. Cab. 8: pl. 771 (1823) ≡ *Platystachys anceps* (Lodd.) Beer, Fam. Bromel.: 80 (1856) ≡ *Vriesea anceps* (Lodd.) Lem., Ill. Hort. 6 (Misc.): 15 (1859) ≡ *Phytarrhiza anceps* (Lodd.) E. Morren, Belgique Hort. 29: 368, pls. 20, 21 (1879).—Type: Adam in Loddiges Hort. s.n.: CGE?, K? (holo), in absence: Original illustration.

= *Tillandsia lineatifolia* Mez, in C.DC., Monogr. phan. 9: 686 (1896).—Type: Fendler 2447: GOET (holo).

Josemania cyanea (Linden ex K. Koch) Barfuss & W. Till, **nom. prov.** ≡ *Tillandsia cyanea* Linden ex K. Koch, Wochenschr. Vereines Beförd. Gartenbaues Königl. Preuss. Staaten 10: 140 (1867).—Type: Linden Hort. s.n.: B? (holo).

= *Tillandsia lindenii* E. Morren, Belgique Hort. 19: 321, pl. 18 (Nov 1869), as „*lindeni*", nom. illeg., non Regel (Mar 1869) ≡ *Vriesea lindenii* (E. Morren) Lem., Ill. Hort. 16: pl. 610 (1869) , nom. illeg. ≡ *Tillandsia morreniana* Regel, Gartenflora 19: 41 (1870) ≡ *Wallisia lindenii* (E. Morren) E. Morren, Belgique Hort. 20: 102 (1870), nom. illeg. ≡ *Phytarrhiza lindenii* (E. Morren) E. Morren, Belgique Hort. 29: 297 (1879), nom. illeg. ≡ *Tillandsia lindenii* [E. Morren] var. *genuina* E. Morren, Gard. Chron., ser. 2, 12: 460 (1879) ≡ *Phytarrhiza lindenii* var. *genuina* E. Morren, Belgique Hort. 29: 297 (1879).—Type: Wallis in Linden Hort. s.n.: LG? (holo).

= *Tillandsia coerulea* Linden ex K. Koch, Wochenschr. Vereines Beförd. Gartenbaues Königl. Preuss. Staaten 13: 197 (1870), nom. nud.

= *Tillandsia lindenii* [E. Morren] *vera* Dombrain, Floral Mag. 11: pl. 44 (1872).—Type: Original illustration.

?= *Tillandsia lindenii* [E. Morren] var. *violacea* hort. ex André, Rev. Hort. 58: 61 (1886).—Type: not indicated.

= *Tillandsia lindenii* [E. Morren] *superba rosea* Dauthenay, Rev. Hort. 70: 539 (1898), nom. illeg.—Type: not indicated.

= *Tillandsia lindenii* [E. Morren] *vera superba* Duval, Gartenwelt 5: 164, Fig. (1901), nom. illeg.—Type: not indicated.

Josemania lindenii (Regel) Barfuss & W. Till, **nom. prov.** ≡ *Tillandsia lindenii* Regel, Index Seminum Hort. Bot. Petrop. "1868": 92 (Mar 1869) ≡ *Wallisia lindenii* (Regel) E. Morren, Belgique Hort. 20: 97 (1870), nom. illeg.—Type: Wallis in St. Petersburg Hort. s.n.: LE (holo).

?= *Tillandsia lindeniana* Regel, Gartenflora 18: 193, pl. 619 (Jul 1869).—Type: Linden Hort. s.n.: LE? (holo).

= *Tillandsia lindenii* [E. Morren] var. *luxurians* E. Morren, Belgique Hort. 21: 289, pls. 20, 21 (1871) ≡ *Phytarrhiza lindenii* var. *luxurians* E. Morren (E. Morren), Belgique Hort. 29: 299 (1879) ≡ *Tillandsia lindenii* [E. Morren] var. *luxurians* (E. Morren) L.B. Sm., Contr. U. S. Natl. Herb. 29: 494 (1951), comb. illeg. superfl. ≡ *Tillandsia lindenii* [E. Morren] var. *abundans* L.B. Sm., Phytologia 20: 166 (1970), nom. nov. superfl.—Type: Belgique Hort. 21: pls. 20, 21 (1871).

= *Phytarrhiza lindenii* var. *koutsinskyana* E. Morren, Belgique Hort. 30: 80 (1880) ≡ *Tillandsia lindenii* [E. Morren] var. *koutsinskyana* (E. Morren) L.B. Sm., Contr. U. S. Natl. Herb. 29: 494 (1951).—Type: Warsaw Hort. s.n.: LG?, WA? (holo).

?= *Tillandsia lindenii* [E. Morren var.] *latispatha* Van Houtte ex André, Rev. Hort. 60: 201 (1888).—Type: Van Houtte s.n.: K? (holo).

= *Tillandsia lindenii* [E. Morren] var. *duvalii* Duval ex André, Rev. Hort. 71: 516 (1899) ≡ *Tillandsia lindenii* [Regel] var. × *duvalii* (Duval ex André) L.B. Sm., Contr. U. S. Natl. Herb. 29: 493 (1951) ≡ *Tillandsia lindenii* [Regel] var. *duvaliana* L.B. Sm., Phytologia 20: 166 (1970), nom. nov. superfl.—Type: Duval Hort. s.n.: ?

= *Tillandsia lindenii* Hasack, Möllers Deutsche Garten-Z. 15: 93, Fig. (1900) non Regel (1869), nom. illeg.—Type: Original illustration.

= *Tillandsia* × *duvali* Duval, Gartenwelt 5: 164, Fig. (1901).—Type: Duval Hort. s.n.: ? (holo), in absence: Original illustration.

= *Tillandsia lindenii* [Regel] var. *caeca* D. Barry, Bromeliad Soc. Bull. 12: 5 (1962) ≡ *Tillandsia lindenii* [Regel] var. × *caeca* D. Barry, in Smith & Downs, Fl. Neotrop. 14(2): 846 (1977).—Type: Barry Hort. s.n.: US (holo).

Josemania pretiosa (Mez) Barfuss & W. Till, **nom. prov.** ≡ *Tillandsia pretiosa* Mez, Repert. Spec. Nov. Regni Veg. 16: 78 (1919).—Type: Sodiro 171/39: B (holo).

= *Tillandsia cyanea* var. *elatior* L.B. Sm., Phytologia 5: 181 (1955).—Type: Fagerlind & Wibom 1947: S (holo).

Josemania umbellata (André) Barfuss & W. Till, **nom. prov.** ≡ *Tillandsia umbellata* André, Rev. Hort. 58: 60, pl. (1886).—Type: André K-317: K (holo).

= *Tillandsia lindenii* [E. Morren] var. *regeliana* E. Morren, Belgique Hort. 20: 225, pl. 12 (1870) ≡ *Phytarrhiza lindenii* var. *regeliana* (E. Morren) E. Morren, Belgique Hort. 29: 298 (1879).—Type: Wallis s.n.: LG? (holo).

= *Tillandsia lindenii* [E. Morren] var. *major* Dombrain, Floral Mag. 10: pl. 529 (1871) ≡ *Tillandsia lindenii* [E. Morren] var. *intermedia* E. Morren, Rev. Hort. 50: 390 (1878), nom. nov. superfl. ≡ *Phytarrhiza lindenii* var. *intermedia* E. Morren, Belgique Hort. 29: 298 (1879), nom. illeg.—Type: Veitch Hort. s.n.: ? (holo), in absence: Original illustration.

?= *Vriesea violacea* hort. ex Houllet, Rev. Hort. 44: 230 (1872), nom. nud.

= *Tillandsia lindenii* [E. Morren] var. *rutilans* Linden ex Houllet, Rev. Hort. 44: 230 (1872), nom. nud.

= *Tillandsia lindenii* [E. Morren] var. *tricolor* André, Ill. Hort. 24: 190 (1877) ≡ *Tillandsia cyanea* var. *tricolor* (André) L.B. Sm., Contr. U. S. Natl. Herb. 29: 491 (1951).—Type: André 4040: K (holo).

= *Tillandsia lindenii* [E. Morren var.] *splendida* Carrière, Rev. Hort. 54: 12, pl. (1882).—Type: Thibaut & Keteleer Hort s.n.: ? (holo).

?= *Tillandsia lindenii* [E. Morren] var. *violacea* hort. ex André, Rev. Hort. 58: 61 (1886), nom. nud.

= *Tillandsia lindenii* [E. Morren] *vera major* Duval, Gartenwelt 5: 164, Fig. (1901), nom. illeg.—Type: Original illustration.

5. *Lemeltonia* Barfuss & W. Till, **nom. prov.**—Type: *Tillandsia dodsonii* L.B. Sm.

A genere *Tillandsia* L. s.str. filamentis basi connatis, stigmate coralliforme et ovulis obtusis claviformibusque, seminibus cum endostomio breviter cylindrico et embryone cum radicula plusminusve distincta differt. Petala alba vel rariter flavescentia.

– *Tillandsia* subg. *Phytarrhiza* (Vis.) Baker p.p., typo excluso.

Plants acaulescent or rarely caulescent; leaves very narrowly triangular, not forming a tank rosette; inflorescence green or brown, lax with the often fragrant flowers mostly spreading; petals white or rarely yellowish; stamens and style deeply included within the corolla, filaments connate among themselves at least at the base; stigma coralliform (Brown & Gilmartin 1989b); ovules clavate, obtuse; seeds with a short-cylindric endostome (type d, Groß 1988) and embryo with the radicule ± separated (type b, Groß 1988).

Named after Elton Martinez Carvalho Leme (1960–) from Rio de Janeiro, Brazil, leading authority of Brazilian Bromeliaceae.

Lemeltonia acosta-solisii (Gilmartin) Barfuss & W. Till, **nom. prov.** ≡ *Tillandsia acosta-solisii* Gilmartin, Phytologia 16: 160 (1968).—Type: Teuscher 2275-56: US (holo).

Lemeltonia cornuta (Mez & Sodiro) Barfuss & W. Till, **nom. prov.** ≡ *Tillandsia cornuta* Mez & Sodiro, Bull. Herb. Boissier, sér. 2, 5: 106 (1905).—Type: Sodiro 171/42: B (holo).

Lemeltonia dodsonii (L.B. Sm.) Barfuss & W. Till, **nom. prov.** ≡ *Tillandsia dodsonii* L.B. Sm., Phytologia 28: 32, pl. 2, figs. f, g (1974).—Type: Dodson 5225: US (holo).

Lemeltonia monadelpha (E. Morren) Barfuss & W. Till, **nom. prov.** ≡ *Phytarrhiza monadelpha* E. Morren, Belgique Hort. 32: 168, pl. 7 (1882) ≡ *Tillandsia monadelpha* (E. Morren) Baker, J. Bot. 25: 281 (1887).—Type: Linden Hort. s.n.: LG (holo).

= *Tillandsia graminifolia* Baker, J. Bot. 25: 281 (1887).—Type: Martin s.n.: BM (lecto); Poiteau s.n.: P? (syn); Sagot 859: P (syn); Fendler 828: K (syn); Parker s.n.: K (syn).

= *Catopsis* (*Andrea*) *alba* E. Morren ex Baker, Handb. Bromel.: 192 (1889), nom. nud.

= *Tillandsia monobotrya* Mez, Repert. Spec. Nov. Regni Veg. 16: 77 (1919).—Type: Wercklé 150 = Inst. Costar. 17444: B (holo).

Lemeltonia narthecioides (C. Presl) Barfuss & W. Till, **nom. prov.** ≡ *Tillandsia narthecioides* C. Presl, Reliq. haenk. 1: 125 (1827).—Type: Haenke s.n.: PR (holo).

Lemeltonia scaligera (Mez & Sodiro) Barfuss & W. Till, **nom. prov.** ≡ *Tillandsia scaligera* Mez & Sodiro, Bull. Herb. Boissier, sér. 2, 5: 107 (1905).—Type: Sodiro 171/4: B (holo).

Lemeltonia triglochinoides (C. Presl) Barfuss & W. Till, **nom. prov.** ≡ *Tillandsia triglochinoides* C. Presl, Reliq. haenk. 1: 125 (1827).—Type: Haenke s.n.: PR (holo).
= *Tillandsia hartwegiana* Brongn. ex Baker, Handb. Bromel.: 171 (1889), nom. nud.

6. *Racinaea* M.A. Spencer & L.B. Sm., Phytologia 74: 152 (1993).—Type: *Tillandsia cuspidata* L.B. Sm.

6.1. *Racinaea* subg. ***Pseudophytarrhiza*** Barfuss & W. Till, **nom. prov.**—Type: *Tillandsia venusta* Mez & Wercklé.
A subgenere typica petalis latioribus unguiculatibusque et stigmate coralliforme differt.
Petals with broader blade and distinct claw; filaments free; stigma coralliform.
– *Tillandsia* subg. *Phytarrhiza* (Vis.) Baker p.p., typo excluso.

Racinaea hamaleana (E. Morren) Barfuss & W. Till, **nom. prov.** ≡ *Tillandsia hamaleana* E. Morren, Gard. Chron. "1869"(2): 460 (1869) ≡ *Wallisia hamaleana* (E. Morren) E. Morren, Belgique Hort. 20: 97, pl. 5 (1870), nom. illeg. ≡ *Phytarrhiza hamaleana* (E. Morren) E. Morren, Belgique Hort. 29: 297 (1879).—Type: Wallis s.n. in Hort. E. Morren: LG? (holo).
= *Tillandsia commelyna* E. Morren, Belgique Hort. 20: 97 (1870), nom. nud.
= *Tillandsia platypetala* Baker, J. Bot. 26: 46 (1888).—Type: Hartweg s.n.: K (holo).
= *Tillandsia nubis* Gilmartin, Phytologia 16: 161 (1968).—Type: Naundorff s.n.: US (holo).

Racinaea venusta (Mez & Wercklé) Barfuss & W. Till, **nom. prov.** ≡ *Tillandsia venusta* Mez & Wercklé, Bull. Herb. Boissier, sér. 2, 5: 108 (1905).—Type: Wercklé Bromel. Costar. 95: B (holo).

6.2. *Racinaea* subg. ***Racinaea***.
= *Tillandsia* subg. *Pseudo-Catopsis* Baker, Handb. Bromel.: 157, 192 (Aug–Oct 1889).—Type: *Tillandsia parviflora* Ruiz & Pav.! (Baker 1889: 192).
= *Tillandsia* sect. *Pseudo-Catopsis* André, Bromel. Andr.: 62, 66 (Sept–Dec 1889), nom. illeg. (Art. 53.4).—Lectotype (Smith & Downs, 1977: 670): *Tillandsia ropalocarpa* André.
– *Tillandsia* subg. *Phytarrhiza* (Vis.) Baker p.p., typo excluso.
Mesophytic, usually acaulescent; petals free and unappendaged; filaments and anthers free; pollen aperture diffuse or with insulae; stigma simple-erect or slightly conduplicate-spiral, rarely coralliform.

Racinaea dyeriana (André) Barfuss & W. Till, **nom. prov.** ≡ *Tillandsia dyeriana* André, Énum. Bromél. 1888: 8 (13 Dec 1888).—Type: André 4256: K (holo), NY (iso).
= *Tillandsia rutschmannii* Rauh, Trop. Subtrop. Pflanzenwelt 12: 5, figs. 1–3, [4a], 5 (1974).—Type: Naundorff s.n. = Hort. Bot. Heidelberg 31701: HEID (holo), US (iso).

7. *Rothowia* Barfuss & W. Till, **nom. prov.**—Type: *Tillandsia wagneriana* L.B. Sm.
A genere *Tillandsia* L. s.str. stigmate obconico typo laminis-convolutis simili, ovulis obtusis vel subobtusis anguste subcylindrico-claviformibus, seminibus cum endostomio conico vel cylindrico et embryone cum radicula plusminusve distincta differt. Petala caerulea.
– *Tillandsia* subg. *Phytarrhiza* (Vis.) Baker p.p., typo excluso.

Plants acaulescent; leaves lingulate, acute, with a distinct leaf-sheath, forming a tank-rosette; inflorescence pink, once to twice branched, rhachis ± alate, floral bracts glabrous; petals blue; stamens and style deeply included within the corolla; filaments lingulate, narrowed at the apex, anthers basifixed; stigma obconic, resembling the convolute-blade type; ovules slenderly cylindric, obtuse or subobtuse; seeds with a conical or cylindrical endostome (type e, Groß 1988) and embryo with a ± distinct radicule (type b, Groß 1988).
Named after Ronald Thomas Wagner (1939–1962).

Rothowia laxissima (Mez) Barfuss & W. Till, **nom. prov.** ≡ *Tillandsia laxissima* Mez, Bull. Herb. Boissier, sér. 2, 5: 108 (1905).—Type: Bang 2301 p.p.: B (holo), G, GH, LE, M, MO, NY, PH, US, W, WU (iso).

Rothowia laxissima var. ***moorei*** (H. Luther) Barfuss & W. Till, **nom. prov.** ≡ *Tillandsia laxissima* var. *moorei* H. Luther, Selbyana 20(1): 13 (1999).—Type: Moore s.n.: SEL (holo).

Rothowia platyrhachis (Mez) Barfuss & W. Till, **nom. prov.** ≡ *Tillandsia platyrhachis* Mez, in C.DC., Monogr. phan. 9: 848 (1896).—Type: Kalbreyer 1328: K (holo).
= *Tillandsia platyrhachis* var. *alba* Rauh & Hirtz, Trop. Subtrop. Pflanzenwelt 53: 11, figs. 5–7 (1985).—Type: Rauh & Hirtz 37571: HEID (holo).
= *Tillandsia platyrhachis* var. *magnifica* Rauh & von Bismarck, Trop. Subtrop. Pflanzenwelt 53: 6, figs. 2–5 (1985).—Type: Rauh & von Bismarck 66107: HEID (holo).

Rothowia wagneriana (L.B. Sm.) Barfuss & W. Till, **nom. prov.** ≡ *Tillandsia wagneriana* L.B. Sm., Phytologia 9: 254, pl. 4, figs. 1–3 (1963).—Type: Moore 310: US (holo).

8. *Tillandsia* L., Sp. pl.: 286 (1753).—Type: *Tillandsia utriculata* L.
Meso- to xerophytic, acaulescent to caulescent; petals free, usually not appendaged; filaments and anthers free; pollen aperture diffuse, with insulae or operculum, or of the *Alcantarea* type; stigma simple-erect or conduplicate-spiral, rarely of the convolute-blade type, seeds of various endostome types (Groß, 1988: e–n) and embryo types (Groß, 1988: b–g).

8.1. *Tillandsia* subg. ***Anoplophytum*** (Beer) Baker, J. Bot. 25: 212 (1887) ≡ *Anoplophytum* Beer, Flora 37: 346 (1854) ≡ *Tillandsia* sect. *Anoplophytum* (Beer) Griseb., Fl. Brit. W. I.: 597 (1864).—Type: *Tillandsia stricta* Sol. ex Sims.
= *Tillandsia* sect. *Eriophyllum* K. Koch, Index Seminum Hort. Bot. Berol. "1873", App. 4: 1 (1874), as "*Eriophorum*".—*Lectotype* (proposed): *Tillandsia selloa* K. Koch. (= *Tillandsia linearis* Vell.).

8.2. *Tillandsia* subg. ***Diaphoranthema*** (Beer) Baker, J. Bot. 16: 236 (1878) ≡ *Diaphoranthema* Beer, Flora 37: 349 (1854) ≡ *Tillandsia* sect. *Diaphoranthema* (Beer) K. Koch, Index Seminum Hort. Bot. Berol. "1873", App. 4: 1 (1874).—Type: *Renealmia recurvata* L.
= *Dendropogon* Raf., Neogenyton: 3 (1825) ≡ *Tillandsia* sect. *Strepsia* Nutt., Gen. N. Amer. pl. 1: 208 (1818) ≡ *Strepsia* (Nutt.) Steud., Nomencl. Bot. ed. 2.2: 645 (1841), nom. illeg.—Type: *Renealmia usneoides* L.

8.3. *Tillandsia* subg. ***Phytarrhiza*** (Vis.) Baker, J. Bot. 25: 212, 214 (1887) ≡ *Phytarrhiza* Vis., Mem. R. Ist. Venet. Sci. 5: 340, pl. (1855) ≡ *Tillandsia* sect. *Phytarrhiza* (Vis.) K. Koch, Index Seminum Hort. Bot. Berol. "1873", App. 4: 1 (1874).—Type: *Tillandsia duratii* Vis.

8.4. ***Tillandsia*** subg. ***Pseudalcantarea*** Mez, in Engl., Pflanzenr. 4.32: 437, 455 (1935).—Type: *Platystachys viridiflora* Beer.

8.5. ***Tillandsia*** subg. ***Pseudovriesea*** Barfuss & W. Till, **nom. prov.**—Type: *Tillandsia tequendamae* André.

Folia anguste triangularia, xeromorphica, dense lepidota, petala plerumque biappendiculata, saepe bicoloria, rariter marginibus crenulatis, stigma laminis conduplicato-spiralibus.

= *Tillandsia* sect. *Trianisandra* André, Bromel. Andr.: 63 (1889).—Type: *Tillandsia heterandra* André.

− *Tillandsia* subg. *Allardtia* A. Dietr. p.p., typo excluso.—studied taxa: *T. myriantha* Baker, *T. barthlottii* Rauh.

− *Tillandsia* subg. *Tillandsia* p.p., typo excluso.—studied taxa: *T. spathacea* Mez & Sodiro.

− *Vriesea* Lindl. p.p., typo excluso.—studied taxa: xerophytic, grey-leaved *Vriesea* spp. (see Appendix for Figure 2 and supplementary data for all spp. included).

Leaves narrowly triangular, xeromorphic, densely lepidote, petals usually with two basal appendages, often two-colored, sometimes with crenulated margins, stigma usually conduplicate-spiral, rarely conduplicate-erect, pollen with diffuse sulcus area; seeds with an elongate-cylindric endostome (type f, Groß, 1988), embryo of type (b–) or ± f (Groß, 1988).

8.6. ***Tillandsia*** subg. ***Tillandsia*** ≡ *Caraguata* Plum. ex Adans., Fam. pl. 2: 67, 532 (Jul–Aug 1763), nom. illeg. (Art. 52.1).—Type: *Tillandsia utriculata* L.

= *Renealmia* L., Sp. pl.: 286 (1753), nom. rejic. vs. *Renealmia* L. f. (1782).—Type: *Renealmia paniculata* L.

= *Bonapartea* Ruiz & Pav., Fl. peruv. 3: 38, pl. 262 (1802) ≡ *Misandra* F. Dietr., Nachtr. vollst. Lex. Gärtn. 5: 102 (1819) non Comm. ex Juss. (1789: Haloragaceae), nom. illeg. ≡ *Acanthospora* Spreng., Syst. veg. 2: 25 (1825), nom. illeg. ≡ *Buonapartea* Sweet, Hort. brit. ed. 3: 706 (1839), orth. var.—Type: *Bonapartea juncea* Ruiz & Pav.

= *Allardtia* A. Dietr., Berliner Allg. Gartenzeitung 20: 241 (1852) ≡ *Platystachys* K. Koch, Index Seminum Hort. Bot. Berol. "1854", App.: 11 (1855), nom. illeg. ≡ *Tillandsia* sect. *Allardtia* (A. Dietr.) E. Morren, Belgique Hort. 27: 272 (1877) ≡ *Tillandsia* subg. *Allardtia* (A. Dietr.) Baker, J. Bot. 26: 40 (1888).—Type: *Allardtia cyanea* A. Dietr. (≡ *Tillandsia guatemalensis* L.B. Sm.).

= *Platystachys* Beer, Fam. Bromel.: 18, 80 (1856), nom. illeg. (Art. 53, Note 1) ≡ *Tillandsia* sect. *Platystachys* (K. Koch) Benth. & Hook. f., Gen. pl. 3(2): 670 (1883) ≡ *Tillandsia* subg. *Platystachys* (K. Koch) Baker, J. Bot. 25: 212, 236 (1887).—Lectotype (Smith & Downs, 1977: 668): *Tillandsia setacea* Sw.

= *Pityrophyllum* Beer, Fam. Bromel.: 17, 79 (1856) ≡ *Tillandsia* sect. *Pityrophyllum* (Beer) K. Koch, Index Seminum Hort. Bot. Berol. "1873", App. 4: 1 (1874) ≡ *Tillandsia* subg. *Pityrophyllum* (Beer) Baker, J. Bot. 26: 39 (1888).—Type: *Tillandsia ionantha* Planch.

= *Vriesea* sect. *Cylindrostachys* Wittm., in Engl. & Prantl, Nat. Pflanzenfam. II.4: 59 (1888) ≡ *Vriesea* subg. *Cylindrostachys* (Wittm.) Harms, in Engl., Nat. Pflanzenfam. ed. 2, 15a: 124 (1930).—Type: *Vriesea malzinei* E. Morren.

− *Tillandsia* subg. *Pseudalcantarea* Mez p.p., typo excluso.—studied taxa: *T. paniculata* (L.) L.

− *Vriesea* sect. *Xiphion* (E. Morren) Wawra ex Wittm. p.p., typo excluso.—studied taxa: *V. malzinei* E. Morren.

8.7. *Tillandsia* subg. ***Viridantha*** (Espejo) Barfuss & W. Till, **nom. prov.** ≡ *Viridantha* Espejo, Acta Bot. Mex. 60: 27 (2002).—Type: *Tillandsia plumosa* Baker.

= *Viridantha* sect. *Caulescens* Espejo, Acta Bot. Mex. 60: 31 (2002).—Type: *Tillandsia tortilis* Klotzsch ex Baker.

− *Tillandsia* subg. *Allardtia* A. Dietr. p.p., typo excluso.—studied taxa: *Tillandsia plumosa* complex, *Tillandsia tectorum* complex (see Appendix for Figure 2 and supplementary data for all spp. included).

− *Tillandsia* subg. *Anoplophytum* p.p., typo excluso.—studied taxa: *T. heteromorpha* Mez.

− *Tillandsia* subg. *Tillandsia* p.p., typo excluso.—studied taxa: *T. lepidosepala* L.B Sm.

8.8. *Biflora* clade.

Name giving taxon: *Tillandsia biflora* Ruiz & Pav.

− *Tillandsia* subg. *Allardtia* A. Dietr. p.p. majore, typo excluso (see Appendix for Figure 2 and supplementary data for all spp. included).

− *Tillandsia* subg. *Pseudalcantarea* Mez p.p., typo excluso.—studied taxa: *T. baliophylla* Harms.

– *Tillandsia* subg. *Anoplophytum* (Beer) Baker p.p., typo excluso.—studied taxa: *T. macbrideana* L.B. Sm., *T. pseudomacbrideana* Rauh.

− *Tillandsia* L. subg. *Tillandsia* p.p., typo excluso.—studied taxa: *T. deppeana* Steud., *T. imperialis* E. Morren ex Roezl, *T. multicaulis* Steud.

8.9. *Gardneri* clade.

Name giving taxon: *Tillandsia gardneri* Lindl.

– *Tillandsia* subg. *Anoplophytum* (Beer) Baker p.p., typo excluso.—studied taxa: *T. brachyphylla* Baker, *T. gardneri* Lindl., *T. globosa* Wawra.

8.10. *Purpurea* clade.

Name giving taxon: *Tillandsia purpurea* Ruiz & Pav.

– *Tillandsia* subg. *Phytarrhiza* (Vis.) Baker p.p., typo excluso.—studied taxa: *T. aurea* Mez, *T. cacticola* L.B. Sm., *T. purpurea* Ruiz & Pav., *T. straminea* Kunth.

8.11. *Rauhii* clade.

Name giving taxon: *Tillandsia rauhii* L.B. Sm.

− *Tillandsia* L. subg. *Tillandsia* p.p., typo excluso.—studied taxa: *T. ecarinata* L.B. Sm., *T. ferreyrae* L.B. Sm., *T. rauhii* L.B. Sm., *T. teres* L.B. Sm..

8.12. *Xiphioides* clade.

Name giving taxon: *Tillandsia xiphioides* Ker Gawl.

– *Tillandsia* subg. *Anoplophytum* (Beer) Baker p.p., typo excluso.—studied taxa: xerophytic, Andean spp. (see Appendix for Figure 2 and supplementary data for all spp. included).

− *Tillandsia* subg. *Allardtia* A. Dietr. p.p., typo excluso.—studied taxa: xerophytic, Andean spp. (see Appendix for Figure 2 and supplementary data for all spp. included).

8.13. Incertae sedis.

The following taxa are currently unclassified within *Tillandsia* L.: *T. albertiana* Verv., *T. australis* Mez, *T. disticha* Kunth, *T. edithae* Rauh, *T. esseriana* Rauh & L.B. Sm., *T. nana* Baker, *T. pseudomicans* Rauh, *T. sphaerocephala* Baker.

Tribe **Vrieseeae** W. Till & Barfuss, Amer. J. Bot. 92: 348 (2005).—Type: *Vriesea* Lindl.
Ovary for a small part inferior, capsule septicidal; petal appendages usually present; stigma mainly of the convolute-blade and cupulate types, less often of the tubolaciniate, conduplicate-erect, conduplicate-patent, conduplicate-spiral, or simple-erect types; seeds with a flight apparatus of pseudohairs at the micropylar end; pollen mainly with exine fragments at the aperture (insulae type) or with solid aperture margins (*Alcantarea* type), less often with a diffuse aperture.

Subtribe **Cipuropsidinae** Barfuss & W. Till, **nom. prov.**—Type: *Cipuropsis* Ule.
A subtribu typica ovulis obtusis vel subobtusis et stigmatibus simplicibus-erectis, condplicato-spialibus vel cupulatis papillosisque differt.
Ovules obtuse or subobtuse, stigma of the simple-erect, conduplicate-spiral, or cupulate types with papillae (Brown & Gilmartin 1989b).

9. *Cipuropsis* Ule, Verh. Bot. Vereins Prov. Brandenburg 48: 148 (1907).—Type: *Cipuropsis subandina* Ule.
– *Tillandsia* subg. *Allardtia* A. Dietr. p.p., typo excluso.—studied taxa: *T. amicorum* I. Ramírez & Bevil.
– *Vriesea* sect. *Xiphion* (E. Morren) Wawra ex Wittm. p.p., typo excluso.—studied taxa: *V. zamorensis* (L.B. Sm.) L.B. Sm.
Petals connate for 25% into a common tube, filaments connate for 5 mm with the petals like the petals themselves, petal appendages present; seeds with an endostome of type c (Groß, 1988) and embryo of type a (Groß, 1988).

Cipuropsis amicorum (I. Ramírez & Bevil.) Barfuss & W. Till, **nom. prov.** ≡ *Tillandsia amicorum* I. Ramírez & Bevil., Acta Bot. Venez. 15: 149, pl. 1 (1989).—Type: Rutkis 452: VEN (holo); Steyermark, Bu[n]ting & Wessels-Boer 100258: MO, VEN (para); Carreño s.n.: VEN (para); Steyermark & Wessels-Boer 100448: MO, VEN (para).

Cipuropsis zamorensis (L.B. Sm.) Barfuss & W. Till, **nom. prov.** ≡ *Tillandsia zamorensis* L.B. Sm., Phytologia 4: 213, pl. 1, figs. 3–5 (1953) ≡ *Vriesea zamorensis* (L.B. Sm.) L.B. Sm., Phytologia 20: 174 (1970).—Type: Scolnik 1500: NY (holo).

10. *Mezobromelia* L.B. Sm., Proc. Amer. Acad. Arts 70: 151 (1935).—Type: *Mezobromelia bicolor* L.B. Sm.
– *Tillandsia* subg. *Allardtia* A. Dietr. p.p., typo excluso.—studied taxa: ?*T. buseri* Mez, *T. schimperiana* Wittm.
– *Vriesea* sect. *Xiphion* (E. Morren) Wawra ex Wittm. p.p., typo excluso.—studied taxa: *V. rubrobracteata* Rauh.
Mesophytic, acaulescent; flower arrangement distichous or polystichous; petals connate/conglutinate and appendaged; filaments free, anthers connate; pollen with diffuse aperture or inaperturate; stigma of the simple-erect type.

Mezobromelia schimperiana (Wittm.) Barfuss & Manzan, **nom. prov.** ≡ *Tillandsia schimperiana* Wittm., Bot. Jahrb. Syst. 11: 67 (1889).—Type: Lehmann XXVI: G (holo).

= *Vriesea rubrobracteata* Rauh, Trop. Subtrop. Pflanzenwelt 27: 13, Fig. 5 (1979).—Type: Rauh 37412: HEID (holo).

?= *Tillandsia buseri* Mez, Bull. Herb. Boissier, sér. 2, 3: 145 (1903).—Type: Langlassé 102a: G (holo), B, K (iso).

11. *Werauhia* J.R. Grant, Trop. Subtrop. Pflanzenwelt 91: 28 (1995) ≡ *Tillandsia* sect. *Xiphion* E. Morren, Belgique Hort. 24: 292 (1874), Lectotype (Smith & Downs, 1977: 1069) ≡ *Vriesea* sect. *Xiphion* (E. Morren) Wawra ex Wittm., in Engl. & Prantl, Nat. Pflanzenfam. II.4: 59 (1888).—Type: *Tillandsia gladioliflora* H. Wendl.

= *Werauhia* sect. *Jutleya* J.R. Grant, Trop. Subtrop. Pflanzenwelt 91: 28, 39.—Type: *Thecophyllum pedicellatum* Mez & Wercklé.

– *Tillandsia* subg. *Allardtia* A. Dietr. p.p., typo excluso.—studied taxa: *T. insignis* Mez.

Mesophytic, usually acaulescent; petals free, mostly appendaged; filaments and anthers free; pollen of the insulae type; stigma cupulate without papillae; seeds with an endostome of type d (Groß, 1988) and embryo of type a, rarely b (Groß, 1988).

12. *Chrysostachys* clade.

Name giving taxon: *Vriesea chrystostachys* E. Morren.

– *Vriesea* sect. *Xiphion* (E. Morren) Wawra ex Wittm. p.p., typo excluso.—studied taxa: *V. chrystostachys* E. Morren, *V. ospinae* H. Luther.

13. *Singularis* clade.

Name giving taxon: *Tillandsia singularis* Mez & Wercklé.

– *Tillandsia* subg. *Allardtia* p.p., typo excluso.—studied taxa: *T. asplundii* L.B. Sm., *T. singularis* Mez & Wercklé.

14. *Splendens* clade.

Name giving taxon: *Vriesea splendens* (Brongn.) Lem.

– *Vriesea* sect. *Vriesea* p.p., typo excluso.—studied taxa: *V. splendens* (Brongn.) Lem..

– *Vriesea* sect. *Xiphion* (E. Morren) Wawra ex Wittm. p.p., typo excluso.—studied taxa: *V. glutinosa* Lindl.

15. Incertae sedis.

The following taxon is currently unclassified within the *Cipuropsis-Mezobromelia* clade: *Vriesea tuerckheimii* (Mez) L.B. Sm.

Subtribe **Vrieseinae** Barfuss & W. Till, **nom. prov.**—Type: *Vriesea* Lindl.

Ovula distincte appendiculata in chalaza, stigmata laminis convolutis, conduplicatis vel tubolaciniatis.

Ovules distinctly appendaged at the chalaza, stigma of the convolute-blade (Brown & Gilmartin 1989b) or tubolaciniate type (Leme & Brown, 2004: *Vriesea plurifolia*) in *Vriesea*, and of the conduplicate-patent (Versieux & Wanderley, 2007; Leme 2007) or rarely of the conduplicate-erect (Leme, 2009: *Alcanatrea roberto-kautskyi*; Leme, unpubl. data: *Alcantarea cerosa*) in *Alcantarea*.

16. *Alcantarea* (E. Morren ex Mez) Harms, Notizbl. Bot. Gart. Berlin-Dahlem 10: 802 (1929) ≡ *Vriesea* subg. *Alcantarea* E. Morren ex Mez, in Mart., Eichler & Urban, Fl. bras. 3(3): 516 (1894), non *Alcantara* Glaz. (1909: Asteraceae), nom nud., nec *Alcantara* Glaz. ex Baroso (1969: Asteraceae), Lectotype (Grant & Zijlstra, 1998: 93) ≡ *Vriesea* Gruppe *„Reginae"* Wawra, Itin. Princip. S. Coburgi 1: 158 (1883) ≡ *Vriesea* sect. *Reginae* (Wawra) Wittm., in Engl. & Prantl, Nat. Pflanzenfam. II.4: 58 (1888).—Type: *Tillandsia regina* Vell.

= *Tillandsia* sect. *Macrocyathus* K. Koch, Index Seminum Hort. Bot. Berol. „1873",App. 4: 1, 6 (1874).—Type: *Tillandsia gigantea* sensu K. Koch (1874) (= *Vriesea glazioviana* Lem.).

Mesophytic, acaulescent; petals free and appendaged; filaments and anthers free; pollen of the *Alcantarea* type; stigma conduplicate-patent, rarely conduplicate-erect.

17. *Vriesea* Lindl., Edwards's Bot. Reg. 29: sub pl. 10 (1843), as "*Vriesia*", nom. conserv. vs. *Hexalepis* Raf., Fl. tellur. 4: 24 (1838) as well as vs. *Vriesea* Hassk., Flora 25 (Beibl.): 27 (1842: Scrophulariaceae) ≡ *Hexalepis* Raf., Fl. tellur. 4: 24 (1838), nom. rejic. vs. *Vriesea* Lindl. ≡ *Tillandsia* sect. *Vriesea* (Lindl.) Griseb., Nachr. Königl. Ges. Wiss. Georg-August-Univ. [1]: 17 (13 Jan 1864) ≡ *Vriesea* ?sect. *Psittacinae* Wawra, Itin. princ. S. Coburgi 1: 158 (1883), nom. illeg. ≡ *Tillandsia* subg. *Vriesea* (Lindl.) Baker, J. Bot. 26: 47 (1888) ≡ *Vriesea* subg. *Euvriese*a Mez, in Mart., Eichler & Urban, Fl. bras. 3(3): 513 (1894) ≡ *Vriesea* sect. *Genuinae* Mez, in Mart., Eichler & Urban, Fl. bras. 3(3): 513 (1894) ≡ *Neovriesia* Britton ex Britton & P. Wilson, Bot. Porto Rico 5: 141 (1923), nom. illeg.—Type: *Tillandsia psittacina* Hook.

= *Tillandsia* sect. *Synandra* K. Koch, Index Seminum Hort. Bot. Berol. "1873", App. 4: 1 (1874).—Type: *Vriesea corallina* Regel.

= *Tillandsia* sect. *Cyathophora* K. Koch, Index Seminum Hort. Bot. Berol. "1873", App. 4: 1 (1874).—Type: *Encholirion saundersii* Carrière.

= *Vriesea* sect. *Platzmanniae* E. Morren, Belgique Hort. 25: 349 (1875).—Type: *Vriesea platzmannii* E. Morren.

= *Vriesea* sect. *Brachystachyae* Wawra, Itin. princ. S. Coburgi 1: 158 (1883).—Type: *Vriesea carinata* Wawra.

= *Vriesea* sect. *Macrostachyae* Wawra, Itin. princ. S. Coburgi 1: 158 (1883).—Type: *Vriesea conferta* Gaudich.

– *Vriesea* subg. *Conostachys* (Griseb.) Mez, in Mart., Eichler & Urban, Fl. bras. 3(3): 516 (1894), typo excluso.—studied taxa: *V. poenulata* (Baker) E. Morren ex Mez, *V. rubida* E. Morren ex Mez (= *V. ventricosa* (Wawra) Mez).

– *Vriesea* sect. *Xiphion* (E. Morren) Wawra ex Wittm. p.p. majore, typo excluso (see Appendix for Figure 2 and supplementary data for all spp. included).

Mesophytic to semi-mesophytic, usually acaulescent; petals free and appendaged; filaments and anthers free; pollen of the insulae type; stigma of the convolute-blade type or tubolaciniate.

Key to the genera of Tillandsioideae

This key must remain provisional as the resolution in the molecular trees is not sufficient in some groups and morphological features are lacking for numerous taxa. However, it demonstrates that the molecular phylogeny can be supported in most cases using ovary, seed, stigma, anther, and petal morphology. The *Cipuropsis-Mezobromelia* clade and *Tillandsia* continue to be critical groups, either because of missing taxa, low phylogenetic resolution, or uncertain application of morphological characters. Therefore, informal clades of the *Cipuropsis-Mezobromelia* clade and subgenera and informal clades of *Tillandsia* are usually not apportioned, except for cases, where differentiating characters have to be utilized for the discrimination of a genus from a subgeneric unit, informal clade or species.

1 Ovary ½–⅔ inferior.—Seeds long appendaged on both ends, chalazal appendage very long, filiform, undivided, micropylar appendage plumose. Flowers polystichous. Pollen with diffuse aperture. Stigma lobes with undulate margins (convolute-blade type).—Lesser Antilles extending to northeastern Venezuela..... ***Glomeropitcairnia***

1* Ovary max. ⅓ inferior to completely superior.—Seeds usually long appendaged only on one end, but chalazal appendage sometimes well developed (e.g., *Alcantarea*, *Tillandsia* p.p., *Vriesea* p.p., *Vriesea tuerckheimii*). Flowers polystichous or distichous **2**

2 Seeds with an undivided micropylar appendage, chalazal multicellular hairs folded at maturity and forming the flight apparatus.—Ovary max. ⅛ inferior to completely superior. Pollen with sharp cut aperture margins (*Catopsis* type). Stigma with erect lobes (simple-erect type), rarely somewhat twisted (tending towards conduplicate-spiral type). Petals usually white and forming a campanulate corolla, rarely yellow and spreading.—Central America and Antilles extending to northern South America and Southeastern Brazil..... ***Catopsis***

2* Seeds with a divided micropylar appendage forming a coma (flight apparatus), chalazal appendage undivided (very rarely divided) and short, or lacking, rarely long and occasionally somewhat divided (*Alcantarea*), not folded at maturity.....**3**

3 Micropylar appendage rather short, about equaling the seed proper, chalazal appendage distinctly larger than the seed proper, sometimes somewhat divided. Petals long, recurved, exposing the long exserted stamens and style.—Pollen with solid aperture margins. Stigma lobes conduplicate and spreading (conduplicate-patent type), rarely not spreading (conduplicate-erect type: *Alcantarea cerosa*, *Alcantarea roberto-kautskyi*), often somewhat twisted around their longitudinal axis.—Endemic to Southeastern Brazil..... ***Alcantarea***

3* Micropylar appendage distinctly longer than the seed proper, chalazal appendage lacking to about half as long as the seed proper, nearly always undivided. Petals forming a tubular or campanulate corolla, at least in the proximal half **4**

4 Petals conglutinated/connate into a distinct tube (distinctly longer than 25%).—Petals white, yellow, or green. Seeds without a distinct chalazal appendage **5**

4* Petals not conglutinated/connate into a distinct tube, completely or at least ¾ free from each other but sometimes connate at the base (max. 25 % of entire length).—Petals violet, pink, red, orange, yellow, green, white, and rarely bicolored. Seeds sometimes with a distinct chalazal appendage up to the length of the seed proper, rarely longer.....**6**

5 Petals with basal appendages *and* anthers forming a tube around the stigma.—Stigma with erect lobes (simple-erect type). Pollen with a diffuse aperture or nearly inaperturate.—Northern Andes extending to the Antilles, the Guianas, and Bolivia .... ***Mezobromelia***

5* Petals without basal appendages *or* anthers not forming a tube around the stigma.—Stigma with erect lobes (simple-erect type) or infundibuliform with undulate margins (convolute-blade type). Pollen with a diffuse aperture or inaperturate.—Andean and Central America, extending to the Antilles, the Guianas, and Eastern Brazil..... ***Guzmania***

6 Stigma lobes fused and forming a cup, margins entire (cupulate type) and without papillae.—Plants mostly forming impounding rosettes. Corolla tubular or campanulate, sometimes zygomorph (stamens then asymmetrically arranged. Pollen with irregular aperture margins and exine fragments in the aperture (insulae type). Seeds with no or only very short chalazal appendage.—Central America extending to Ecuador (and Bolivia). ***Werauhia***

6* Stigma lobes not of the cupulate type without papillae, but rarely resembling cupulate with crenulate (tubolaciniate type) (*Vriesea* p.p.) or papillate (*Vriesea tuerckheimii*) margins..... **7**

7 Stigma with undulate and papillate margins (convolute-blade type), rarely with crenulate margins (tubolaciniate type), plants meso- or semi-mesophytic, petal appendages present.—Corolla tubular or campanulate, stamens and style exserted or included. Petals yellow (often with green tips), cream, or brownish (–red), rarely white, free or rarely short connate into a common tube with the filament bases. Pollen with irregular aperture margins and exine fragments in the aperture (insulae type). Seeds with a distinct chalazal appendage.—Eastern Brazil extending to Venezuela and Peru ..... ***Vriesea***

7* Stigma lobes usually not of the convolute-blade or tubolaciniate types, if rarely resembling convolute-blade then plants xerophytic and petal appendages absent **8**

8 Filaments connate among each other at least at the base but sometimes for nearly their whole length, free from the petals. Stigma coralliform.—Leaves narrowly triangular. Petals white ore yellowish. Ovules clavate, obtuse. Seeds without chalazal appendage.—Central America to Peru extending to eastern Venezuela and the Guianas ..... ***Lemeltonia***

8* Filaments free from each other, but sometimes connate/agglutinated to the petals (e.g., *Cipuropsis* and *Racinaea*). Stigma usually not of the coralliform type, if rarely resembling the coralliform type then filaments free from each other (*Racinaea* subg. *Pseudophytarrhiza* and *Racinaea dyeriana*) **9**

9 Stigma pinnatisect.—Inflorescence undivided, distichous, flat, lanceolate-elliptic in outline. Petals violet, their blades usually strongly enlarged. Leaf bases mostly longitudinally red striped. Ovules slenderly cylindric, obtuse. Seeds without chalazal appendage.—Ecuador and Peru, *Josemania anceps* extending to Guatemala, eastern Venezuela, the Guianas, and northern Brazil ..... ***Josemania***

9* Stigma not pinnatisect..... **10**

10 Stigma obconical and resembling the convolute-blade type, mesophytic.—Leaves lingulate, forming impounding rosettes. Inflorescence compound, pink, rhachis usually alate. Flowers distichous, petals with enlarged blade, violet. Seeds with a minute chalazal appendage only.—Andean, Ecuador to Bolivia ..... ***Rothowia***

10* Stigma not obconical and not resembling the convolute-blade type, if rarely of the convolute-blade type, then plants xerophytic and not forming impounding rosettes (*Tillandsia plumosa* complex)..... **11**

11 Sepals distinctly asymmetric, stigma simple-erect or slightly conduplicate-spiral (both reduction forms of the coralliform type), if subsymmetric then stigma resembling the coralliform type and leaves lingulate (*Racinaea* subg. *Pseudophytarrhiza* and *Racinaea dyeriana*). Seeds without chalazal appendage.—Flowers small and inconspicuous in most species, petals white to yellow, rarely blue (*Racinaea hamaleana*).—Andean, mainly Ecuador, extending to Central America, Bolivia, the Greater Antilles and the Guianas, and Southeastern Brazil ..... ***Racinaea***

11* Sepals symmetric, stigma usually simple-erect or conduplicate-spiral, rarely convolute-bladed (*Tillandsia* subg. *Viridantha* p.p.) or resembling cupulate with papillate margins (*Vriesea tuerckheimii*). Seeds usually with a distinct chalazal appendage..... **12**

12 Petals without basal appendages..... **13**

12* Petals with basal appendages **14**

13 Petals and filaments basally connate *or* leaf sheaths drying silver-gray.—Petals white or yellow .....***Singularis*** clade

13* Petals and filaments basally not connate *and* leaf sheaths not drying silver-gray.—From the southern United Stated to the Antilles, central Argentina and Uruguay with centers of diversity in northern Central America and the northern and central Andes (not further keyed out except for taxa of *Tillandsia* subg. *Pseudovriesea* bearing petal appendages)..... .....***Tillandsia***

14 Leaves narrowly triangular, xeromorphic, densely lepidote.—Petals usually violet, often bicolored with green, sometimes with crenulated margins.—Northern and central Andes extending to Bolivia, Mesoamerica, the Antilles, and eastern Venezuela..... ..... ***Tillandsia*** subg. ***Pseudovriesea*** p.p.

14* Leaves lingulate, usually mesomorphic, not densely lepidote.—Corolla tubular, stamens and style usually included, rarely exserted. Petals white or yellow, rarely red. Pollen with a diffuse aperture..... **15**

15 Plants Andean extending to Venezuela, Trinidad, and the Guianas ....***Cipuropsis***, ***Chrysostachys*** clade, ***Splendens*** clade, ***Vriesea tuerckheimii***

15* Plant from Mexico .....***Tillandsia malzinei***

Conclusions

Much progress has been achieved in exploring phylogenetic relationships of Tillandsioideae by adding nuclear DNA information to the already existing and published plastid DNA data (Barfuss & al., 2005). Most accepted genera are well differentiated by both DNA and morphological data in their actual circumscriptions (*Alcantarea*, *Catopsis*, *Glomeropitcairnia*, *Josemania*, *Lemeltonia*, *Racinaea*, *Rothowia*, *Vriesea*, *Werauhia*). *Guzmania* in its present circumscription is holophyletic, but whether the corolla tube formation (Leins & Erbar, 2010) is a synapomorphic character for all *Guzmania* and different to that of *Mezobromelia* needs to be further tested. Generic definitions of the *Cipuropsis-Mezobromelia* clade need further attention, mostly in adding missing taxa and more DNA data as well as in evaluating morphological characters for defining clades and assessing their phylogenetic position relative to the preliminarily accepted genera *Cipuropsis* and *Mezobromelia*. *Tillandsia* as presently defined is holophyletic according to DNA data, but morphologically still very diverse, which is also demonstrated by the numerous subgenera and informal clades. Although several questions concerning relationships of species of former *Tillandsia* subg. *Phytarrhiza* and *T.* subg. *Pseudalcantarea* are solved, in other subgenera statistical support for their holophyletic origin is still missing. Relationships between clades and subgenera are also mostly unclear and lack statistical support. A solution has to be found for the unclassified species of *Tillandsia*. To get a fully resolved picture of relationships within Tillandsioideae, more nuclear DNA markers and a careful revision of morphological characters already identified as useful for discriminating taxa are still needed.

Acknowledgments

The authors thank Tod F. Stuessy for providing lab space; many botanical gardens and individual people for providing plant material (see "Taxon selection"); the "Botanischer Garten der Universität Wien" (HBV) for cultivating the majority of samples used; Jason Grant for valuable discussions; Eva Maria Mayr for helping with plant collections in Ecuador (2006); and Anton Russell for critically reviewing the manuscript. Financial support was partly provided by the Austrian Science Foundation (FWF) to W. Till (grant no. P13690-BIO), by the Commission for Interdisciplinary Ecological Studies (KIÖS) at the Austrian Academy of Sciences (ÖAW) to W. Till and M.H.J. Barfuss (2007-02), and by the Dept. of Systematic and Evolutionary Botany (Universität Wien).

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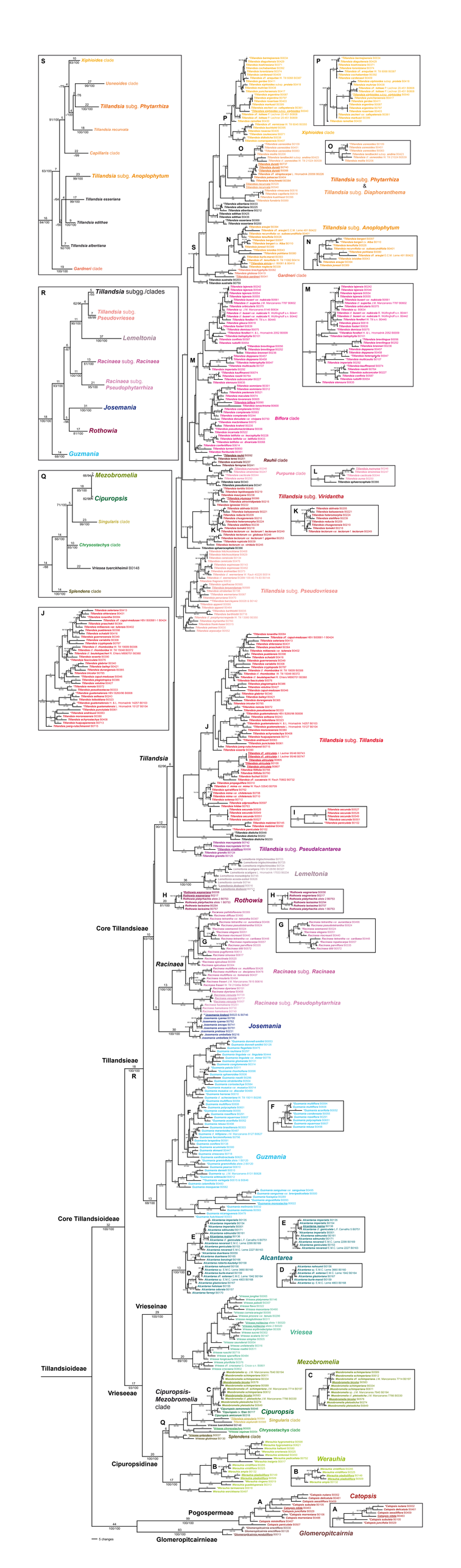
Appendix

Figure 2. (p. 193) Selected equally most-parsimonious phylogram found in total combined analysis supplemented with BP and PP mostly below branches, and branch length is given above branches. A small arrowhead below branches indicates BP and PP positions where space was insufficient. Alternative topologies of terminal branches and backbone relationships from Bayesian analysis are displayed in boxes mostly in front of the corresponding clades or on the left side. Their positions within the complete tree are highlighted by capital letters (A–P: terminals, Q–S: backbones). Relevant type species for each taxonomic unit and name-giving taxa for informal clades are underlined. For samples which are similar to a already described species (aff.), with a vague identification (cf.), or undetermined species (sp.), as well as for clearly polyphyletic species voucher details are given in addition to the DNA reference number. Alternative tree topologies from Bayesian analysis are (1) terminals: A = *Catopsis* p.p., B = *Werauhia* p.p., C = *Mezobromelia*, D and E = *Alcantarea* p.p., F = *Guzmania* p.p., G = *Racinaea* p.p., H = *Rothowia*, I and J = *T.* subg. *Tillandsia* p.p., K = *T.* subg. *Viridantha* p.p., L = *Purpurea* clade, M = *Biflora* clade p.p., N = *T.* subg. *Anoplophytum* p.p., O = *T.* subg. *Diaphoranthema* p.p., P = *Xiphioides* clade; (2) backbones: Q = *Cipuropsis-Mezobromelia* clade, R = *Tillandsieae*, S = *T.* subg. *Anoplophytum* s.l. plus *T*. subg. *Phytarrhiza* and *T*. subg. *Diaphoranthema*.

Supplementary Data. (pp. 195–205) Accessions included in this study arranged in hierarchical, followed by alphabetical order (according to the chapter "Classification system of Tillandsioideae"). Attributes ofamplified fragments of *PHYC* and *PRK* are listed. SNP = single nucleotide polymorphism, SSR = single sequence repeat (homopolymers).


Xiphiolides clade
Usneolides clade
Tillandsia subg. Phytarrhiza
Tillandsia recurvata
Capillaris clade
Tillandsia subg. Anoplophytarum
Tillandsia esseriana
Tillandsia edithae
Tillandsia albertiana
Gardneri clade
Tillandsia subgg./clades
Tillandsia subg. Pseudovriesea
Lemeltonia
Racinaea subg. Racinaea
Racinaea subg. Pseudopothyarrhiza
Josemania
Rothowia
Guzmania
Mezobromelia
Cipuropsis
Singularis clade
Chrysostachys clade
Vriesea tuerckheimii B0148
Spherdens clade
Tillandsia subg. Tillandsia
Tillandsia
Core Tillandsieae
Racinaea
Racinaea subg. Racinaea
Racinaea subg. Pseudophytarrhiza
Josemania
Tillandsieae
Core Tillandsioideae
Alcantarea
Vrieseinae
Vriesea
Tillandsioideae
Vrieseeae
Cipuropsis-Mezobromelia clade
Cipuropsidinae
Cipuropsis
Werauhia
Pogospermeae
Catopsis
Glomeropitcairnieae
Glomeropitcairnia
Tillandsia subg. Phytarrhiza
Tillandsia subg. Diaphoranthema
Tillandsia subg. Anoplophytarum
Gardneri clade
Bifora clade
Rauhii clade
Purpurea clade
Tillandsia subg. Viridantha
Tillandsia subg. Pseudovriesea
Tillandsia subg. Tillandsia
Tillandsia subg. Pseudalcantarea
Lemeltonia
Rothowia
Core Tillandsieae
Racinaea
Racinaea subg. Racinaea
Racinaea subg. Pseudophytarrhiza
Josemania
Guzmania
Core Tillandsioideae
Alcantarea
Vrieseinae
Vriesea
Mezobromelia
Cipuropsis-Mezobromelia clade
Cipuropsis
Singularis clade
Spherdens clade
Werauhia
Catopsis
Glomeropitcairnia
5 changes

		PHYC			*PRK*							
Taxon	**DNA no.**	**SNPs**	**Geno types**	**Length**	**SNPs**	**Indels**	**SSR**	**Geno types**	**Consensus length**	**Allele 1 length (a1)**	**Allele 2 length (a2)**	**postulated ploidy level**
Bromeliaceae tribe ***Glomeropitcairnieae*** Harms												
Glomeropitcairnia (Mez) Mez												
Glomeropitcairnia erectiflora Mez	B0030	1	2	1177	7	0	0	2	1198	1198	1198	2n = 2x
Glomeropitcairnia erectiflora Mez	B0128	0	1	1177	0	0	0	1	1198	1198	1198	2n = 2x
Glomeropitcairnia penduliflora (Griseb.) Mez	B0013	0	1	1177	0	0	0	1	1205	1205	1205	2n = 2x
Bromeliaceae tribe ***Pogospermeae*** Brongn.												
Catopsis Griseb.												
Catopsis delicatula L.B. Sm.	B0461	3	2	1177	0	0	0	1	927	927	927	2n = 2x
Catopsis juncifolia Mez & Wercklé ex Mez	B0029	0	1	1177	0	0	0	1	975	975	975	2n = 2x
Catopsis minimiflora Matuda	B0467	0	1	1177	7	1	0	2	1005	1005	1004	2n = 2x
Catopsis morreniana Mez	B0106	0	1	1177	3	0	0	2	969	969	969	2n = 2x
Catopsis morreniana Mez	B0488	0	1	1177	4	0	0	2	969	969	969	2n = 2x
Catopsis nitida (Hook.) Griseb.	B0463	0	1	1177	0	0	0	1	968	968	968	2n = 2x
Catopsis nutans (Sw.) Griseb.	B0002	0	1	1177	0	0	0	1	927	927	927	2n = 2x
Catopsis paniculata E. Morren	B0507	0	1	1177	0	0	0	1	892	892	892	2n = 2x
Catopsis sessiliflora (Ruiz & Pav.) Mez	B0459	1	2	1177	6	0	0	2	927	927	927	2n = 2x
Catopsis subulata L.B. Sm.	B0105	2	2	1177	6	0	0	2	969	969	969	2n = 2x
Bromeliaceae tribe ***Tillandsieae*** Rchb.												
Guzmania Ruiz & Pav.												
Guzmania acorifolia (Griseb.) Mez	B0052	0	1	1192	0	0	0	1	1021	1021	1021	2n = 2x
Guzmania acuminata L.B. Sm.	B0300	0	1	1177	1	0	0	2	992	992	992	2n = 2x
Guzmania angustifolia (Baker) Wittm.	B0093	1	2	1177	9	2	0	2	1050	1050	1018	2n = 2x
Guzmania brasiliensis Ule	B0303	0	1	1177	0	0	0	1	1037	1037	1037	2n = 2x
Guzmania calamifolia André ex Mez	B0483	1	2	1177	1	0	0	2	1033	1033	1033	2n = 2x
Guzmania condensata Mez & Wercklé	B0055	0	1	1192	0	0	0	1	918	918	918	2n = 2x
Guzmania conglomerata H. Luther	B0314	0	1	1177	8	0	0	2	967	967	967	2n = 2x
Guzmania conifera (André) André ex Mez	B0138	2	2	1177	0	0	0	1	993	993	993	2n = 2x
Guzmania coriostachya (Griseb.) Mez	B0584	1	2	1177	5	1	0	2	926	920	926	2n = 2x
Guzmania danielii L.B. Sm.	B0510	1	2	1177	2	0	0	2	1056	1056	1056	2n = 2x
Guzmania donnell-smithii Mez	B0053	0	1	1177	0	0	0	1	1040	1040	1040	2n = 2x
Guzmania donnell-smithii Mez	B0126	0	1	1177	0	0	0	1	1040	1040	1040	2n = 2x
Guzmania ekmanii (Harms) Harms ex Mez	B0447	0	1	1177	0	0	0	1	1026	1026	1026	2n = 2x
Guzmania farciminiformis H. Luther	B0796	1	2	1177	3	0	0	2	1008	1008	1008	2n = 2x
Guzmania flagellata S. Pierce & J.R. Grant	B0475	0	1	1177	0	0	0	1	1035	1035	1035	2n = 2x
Guzmania fusispica Mez & Sodiro	B0289	0	1	1177	0	0	0	1	1050	1050	1050	2n = 2x
Guzmania glomerata Mez & Wercklé	B0131	0	1	1177	0	0	0	1	967	967	967	2n = 2x
Guzmania graminifolia (André ex Baker) L.B. Sm.	B0120	2	2	1177	9	4	1	2	n.u.	1209!	1185!	2n = 2x
Guzmania herrerae H. Luther & W.J. Kress	B0010	1	2	1177	13	1	1	2	1220	1220	1219	2n = 2x
Guzmania hutchisonii (L.B. Sm.) Barfuss & W. Till	B0003	1	2	1177	0	0	0	1	1129	1129	1129	2n = 2x
Guzmania cf. *killipiana* L.B. Sm.	B0627	0	1	1177	8	0	0	2	1020	1020	1020	2n = 2x
Guzmania lingulata (L.) Mez var. *lingulata*	B0444	0	1	1177	0	0	0	1	1003	1003	1003	2n = 2x
Guzmania lingulata var. *minor* (Mez) L.B. Sm. & Pittendr.	B0778	0	1	1177	0	0	0	1	949	949	949	2n = 2x
Guzmania marantoidea (Rusby) H. Luther	B0487	6	2	1177	6	2	0	2	1022	1018	939	2n = 2x
Guzmania melinonis Regel	B0032	0	1	1177	0	0	0	1	1132	1132	1132	2n = 2x
Guzmania melinonis Regel	B0593	1	2	1177	0	0	1	1	1154	1154	1154	2n = 2x
Guzmania monostachia (L.) Rusby ex Mez	B0022	0	1	1177	0	0	0	1	1005	1005	1005	2n = 2x

		PHYC			*PRK*							
Taxon	**DNA no.**	**SNPs**	**Geno types**	**Length**	**SNPs**	**Indels**	**SSR**	**Geno types**	**Consensus length**	**Allele 1 length (a1)**	**Allele 2 length (a2)**	**postulated ploidy level**
Guzmania mosquerae (Wittm.) Mez	B0582	0	1	1180	0	0	0	1	1055	1055	1055	2n = 2x
Guzmania multiflora (André) André ex Mez	B0094	0	1	1177	0	0	0	1	926	926	926	2n = 2x
Guzmania multiflora (André) André ex Mez	B0606	0	1	1177	0	0	1	1	1098	1098	1098	2n = 2x
Guzmania musaica var. *discolor* H. Luther	B0489	0	1	1177	4	0	1	2	1225	1225	1225	2n = 2x
Guzmania musaica (Linden & André) Mez var. *musaica*	B0014	1	2	1177	1	0	1	2	1225	1225	1225	2n = 2x
Guzmania nicaraguensis Mez & C.F. Baker	B0479	0	1	1177	0	0	0	1	1127	1127	1127	2n = 2x
Guzmania patula Mez & Wercklé	B0011	0	1	1177	0	0	0	1	831	831	831	2n = 2x
Guzmania pearcei (Baker) L.B. Sm.	B0610	0	1	1177	0	0	1	1	1232	1232	1232	2n = 2x
Guzmania polycephala Mez & Wercklé ex Mez	B0651	0	1	1177	0	0	0	1	917	917	917	2n = 2x
Guzmania rauhiana H. Luther	B0297	0	1	1177	0	0	0	1	1325	1325	1325	2n = 2x
Guzmania retusa L.B. Sm.	B0498	1	2	1177	0	0	0	1	995	995	995	2n = 2x
Guzmania rhonhofiana Harms	B0096	0	1	1177	0	0	0	1	831	831	831	2n = 2x
Guzmania roezlii (E. Morren) Mez	B0286	0	1	1177	0	0	0	1	884	884	884	2n = 2x
Guzmania roseiflora Rauh	B0291	6	2	1177	1	1	0	2	934	934	928	2n = 2x
Guzmania sanguinea var. *brevipedicellata* Gilmartin	B0500	0	1	1177	3	0	0	2	1050	1050	1050	2n = 2x
Guzmania sanguinea (André) André ex Mez var. *sanguinea*	B0495	2	2	1177	4	0	0	2	1050	1050	1050	2n = 2x
Guzmania cf. *scherzeriana* Mez	B0295	0	1	1177	0	0	1	1	1218	1218	1218	2n = 2x
Guzmania sphaeroidea (André) André ex Mez	B0506	0	1	1177	1	0	0	2	838	838	838	2n = 2x
Guzmania squarrosa (Mez & Sodiro) L.B. Sm. & Pittendr.	B0607	0	1	1177	0	0	0	1	929	929	929	2n = 2x
Guzmania strobilantha (Ruiz & Pav.) Mez	B0504	0	1	1177	0	0	0	1	880	880	880	2n = 2x
Guzmania tarapotina Ule	B0591	7	2	1177	14	2	0	2	1038	?	?	2n = 2x
Guzmania variegata L.B. Sm.	B0015	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Guzmania variegata L.B. Sm.	B0646	8	2	1177	0	0	0	1	1015	1015	1015	2n = 2x
Guzmania virescens (Hook.) Mez	B0718	5	2	1177	3	1	0	2	1035	1035	1029	2n = 2x
Guzmania wittmackii (André) André ex Mez	B0012	0	1	1177	0	0	0	1	1030	1030	1030	2n = 2x
Guzmania xanthobractea Gilmartin	B0623	0	1	1177	0	0	0	1	1033	1033	1033	2n = 2x
Guzmania sp.	B0628	1	2	1177	2	0	0	2	1043	1043	1043	2n = 2x
Josemania Barfuss & W. Till												
Josemania anceps (Lodd.) Barfuss & W. Till	B0741	0	1	1177	0	0	0	1	1048	1048	1048	2n = 2x
Josemania anceps (Lodd.) Barfuss & W. Till	B0781	0	1	1177	0	0	0	1	1048	1048	1048	2n = 2x
Josemania cyanea (Linden ex K. Koch) Barfuss & W. Till	B0780	2	2	1177	4	1	0	2	1049	1049	1048	2n = 2x
Josemania cyanea (Linden ex K. Koch) Barfuss & W. Till	B0782	5	2	1177	6	0	0	2	1049	1049	1049	2n = 2x
Josemania lindenii (Regel) Barfuss & W. Till	B0023	n.d	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Josemania lindenii (Regel) Barfuss & W. Till	B0746	0	1	1177	7	0	0	2	1049	1049	1049	2n = 2x
Josemania pretiosa (Mez) Barfuss & W. Till	B0231	3	2	1177	0	0	0	1	1049	1049	1049	2n = 2x
Josemania umbellata (André) Barfuss & W. Till	B0216	1	2	1177	9	1	1	2	1071	1071	1070	2n = 2x
Josemania umbellata (André) Barfuss & W. Till	B0758	5	2	1177	14	1	1	2	1071	1071	1070	2n = 2x
Lemeltonia Barfuss & W. Till												
Lemeltonia acosta-solisii (Gilmartin) Barfuss & W. Till	B0626	0	1	1177	0	0	1	1	1103	1103	1103	2n = 2x
Lemeltonia cornuta (Mez & Sodiro) Barfuss & W. Till	B0744	0	1	1177	0	0	1	1	1103	1103	1103	2n = 2x
Lemeltonia dodsonii (L.B. Sm.) Barfuss & W. Till	B0016	3	2	1177	0	0	1	1	1137	1137	1137	2n = 2x
Lemeltonia dodsonii (L.B. Sm.) Barfuss & W. Till	B0721	1	2	1177	0	0	1	1	1116	1116	1116	2n = 2x
Lemeltonia monadelpha (E. Morren) Barfuss & W. Till	B0745	0	1	1177	0	0	1	1	1120	1120	1120	2n = 2x
Lemeltonia scaligera (Mez & Sodiro) Barfuss & W. Till	B0234	0	1	1177	9	0	1	2	1119	1119	1119	2n = 2x
Lemeltonia scaligera (Mez & Sodiro) Barfuss & W. Till	B0327	1	2	1177	4	0	1	2	1120	1120	1120	2n = 2x
Lemeltonia triglochinoides (C. Presl) Barfuss & W. Till	B0723	5	2	1177	7	0	1	2	1118	1118	1118	2n = 2x

		PHYC			*PRK*							
Taxon	**DNA no.**	**SNPs**	**Geno types**	**Length**	**SNPs**	**Indels**	**SSR**	**Geno types**	**Consensus length**	**Allele 1 length (a1)**	**Allele 2 length (a2)**	**postulated ploidy level**
Lemeltonia triglochinoides (C. Presl) Barfuss & W. Till	B0724	3	2	1177	0	0	1	1	1126	1126	1126	2n = 2x
Lemeltonia triglochinoides (C. Presl) Barfuss & W. Till	B0725	3	2	1177	4	1	1	2	1125	?	?	2n = 2x
Racinaea M.A. Spencer & L.B. Sm.												
Racinaea subg. ***Pseudophytarrhiza*** Barfuss & W. Till												
Racinaea hamaleana (E. Morren) Barfuss & W. Till	B0251	0	1	1177	2	1	1	2	1122	1122	1121	2n = 2x
Racinaea hamaleana (E. Morren) Barfuss & W. Till	B0730	0	1	1177	6	1	1	2	1118	1118	1119	2n = 2x
Racinaea hamaleana (E. Morren) Barfuss & W. Till	B0749	1	2	1177	8	1	1	2	1122	1122	1118	2n = 2x
Racinaea venusta (Mez & Wercklé) Barfuss & W. Till	B0007	0	1	1177	4	0	1	2	1131	1131	1131	2n = 2x
Racinaea venusta (Mez & Wercklé) Barfuss & W. Till	B0729	2	2	1177	2	2	1	2	1129	?	?	2n = 2x
Racinaea venusta (Mez & Wercklé) Barfuss & W. Till	B0731	0	1	1177	2	2	1	2	1129	?	?	2n = 2x
Racinaea subg. ***Racinaea***												
Racinaea diffusa (L.B. Sm.) M.A. Spencer & L.B. Sm.	B0480	2	2	1177	0	0	0	1	844	844	844	2n = 2x
Racinaea dyeriana (André) Barfuss & W. Till	B0151	0	1	1177	0	0	0	1	953	953	953	2n = 2x
Racinaea dyeriana (André) Barfuss & W. Till	B0456	0	1	1177	4	0	0	2	953	953	953	2n = 2x
Racinaea elegans (L.B. Sm.) M.A. Spencer & L.B. Sm.	B0051	1	2	1177	0	0	0	1	881	881	881	2n = 2x
Racinaea fraseri (Baker) M.A. Spencer & L.B. Sm.	B0547	0	1	1177	0	0	1	1	1144	1144	1144	2n = 2x
Racinaea fraseri (Baker) M.A. Spencer & L.B. Sm.	B0616	0	1	1177	0	0	1	1	1140	1140	1140	2n = 2x
Racinaea insularis (Mez) M.A. Spencer & L.B. Sm.	B0494	0	1	1177	0	0	0	1	1139	1139	1139	2n = 2x
Racinaea multiflora var. *decipiens* (André) M.A. Spencer & L.B. Sm.	B0478	1	2	1177	0	0	1	1	1141	1141	1141	2n = 2x
Racinaea multiflora (Benth.) M.A. Spencer & L.B. Sm. var. *multiflora*	B0426	0	1	1177	0	0	1	1	1141	1141	1141	2n = 2x
Racinaea multiflora var. *tomensis* (L.B. Sm.) M.A. Spencer & L.B. Sm.	B0437	0	1	1177	7	0	0	2	1142	1142	1142	2n = 2x
Racinaea pallidoflavens (Mez) M.A. Spencer & L.B. Sm.	B0369	1	2	1177	5	0	0	2	838	838	838	2n = 2x
Racinaea parviflora (Ruiz & Pav.) M.A. Spencer & L.B. Sm.	B0335	4	2	1177	5	0	0	2	885	885	885	2n = 2x
Racinaea pectinata (André) M.A. Spencer & L.B. Sm.	B0520	0	1	1177	0	0	0	1	920	920	920	2n = 2x
Racinaea pseudotetrantha (Gilmartin & H. Luther) J.R. Grant	B0624	0	1	1177	0	0	0	1	870	870	870	2n = 2x
Racinaea pugiformis (L.B. Sm.) M.A. Spencer & L.B. Sm.	B0613	1	2	1177	0	0	0	1	902	902	902	2n = 2x
Racinaea riocreuxii (André) M.A. Spencer & L.B. Sm.	B0440	3	2	1177	3	0	0	2	885	885	885	2n = 2x
Racinaea ropalocarpa (André) M.A. Spencer & L.B. Sm.	B0057	5	2	1177	9	1	0	2	885	885	881	2n = 2x
Racinaea seemannii (Baker) M.A. Spencer & L.B. Sm.	B0024	1	2	1177	7	3	0	2	869	?	?	2n = 2x
Racinaea sinuosa (L.B. Sm.) M.A. Spencer & L.B. Sm.	B0617	4	2	1177	1	0	0	2	940	940	940	2n = 2x
Racinaea spiculosa (Griseb.) M.A. Spencer & L.B. Sm.	B0099	0	1	1177	0	0	0	1	979	979	979	2n = 2x
Racinaea spiculosa (Griseb.) M.A. Spencer & L.B. Sm.	B0304	0	1	1177	0	0	1	1	1008	1008	1008	2n = 2x
Racinaea tetrantha var. *aurantiaca* (Griseb.) M.A. Spencer & L.B. Sm.	B0496	0	1	1177	0	0	0	1	870	870	870	2n = 2x
Racinaea tetrantha var. *caribaea* (L.B. Sm.) M.A. Spencer & L.B. Sm.	B0448	0	1	1177	0	0	0	1	906	906	906	2n = 2x
Racinaea tetrantha (Ruiz & Pav.) M.A. Spencer & L.B. Sm. var. *tetrantha*	B0367	2	2	1177	5	1	0	2	906	906	904	2n = 2x
Racinaea tilli Manzan. & Gouda	B0572	0	1	1177	3	1	0	2	908	908	906	2n = 2x
Rothowia Barfuss & W. Till												
Rothowia laxissima (Mez) Barfuss & W. Till	B0294	0	1	1177	0	0	1	1	1144	1144	1144	2n = 2x
Rothowia laxissima (Mez) Barfuss & W. Till	B0757	0	1	1177	0	0	1	1	1144	1144	1144	2n = 2x
Rothowia platyrhachis (Mez) Barfuss & W. Till	B0753	2	2	1177	10	3	1	2	n.u.	1149	1143	2n = 2x
Rothowia wagneriana (L.B. Sm.) Barfuss & W. Till	B0058	0	1	1177	0	0	1	1	1149	1149	1149	2n = 2x
Rothowia wagneriana (L.B. Sm.) Barfuss & W. Till	B0217	0	1	1177	0	0	1	1	1149	1149	1149	2n = 2x
Tillandsia L.												
Tillandsia subg. ***Anoplophytum*** (Beer) Baker												
Tillandsia araujei Mez	B0394	9	2	1177	6	0	1	2	1293	1293	1293	2n = 2x

		PHYC			***PRK***							
Taxon	**DNA no.**	**SNPs**	**Geno types**	**Length**	**SNPs**	**Indels**	**SSR**	**Geno types**	**Consensus length**	**Allele 1 length (a1)**	**Allele 2 length (a2)**	**postulated ploidy level**
Tillandsia aff. *araujei* Mez	B0422	0	1	1177	2	0	1	2	1112	1112	1112	2n = 2x
Tillandsia bergeri Mez	B0097	7	2	1177	0	0	0	1	1058	1058	1058	2n = 2x
Tillandsia bergeri Mez cv. Alba	B0110	2	2	1177	0	0	1	1	1089	1089	1089	2n = 2x
Tillandsia burle-marxii Ehlers	B0393	0	1	1177	0	0	1	1	1133	1133	1133	2n = 2x
Tillandsia ixioides Griseb.	B0043	0	1	1177	1	0	1	2	1129	1129	1129	2n = 2x
Tillandsia jonesii Strehl	B0389	3	2	1177	13	1	1	2	1113	1113	1109	2n = 2x
Tillandsia neglecta E. Pereira	B0339	9	2	1177	3	0	1	2	1120	1120	1120	2n = 2x
Tillandsia pohliana Mez	B0080	0	1	1177	0	0	0	1	990	990	990	2n = 2x
Tillandsia recurvifolia var. *subsecundifolia* (W. Weber & Ehlers) W. Till	B0401	1	2	1177	4	0	1	2	1131	1131	1131	2n = 2x
Tillandsia stricta var. *albifolia* H. Hrom. & Rauh	B0410	1	2	1177	2	0	1	2	1113	1113	1113	2n = 2x
Tillandsia stricta Sol. ex Sims var. *stricta*	B0081	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d	n.d.	n.d.	n.d.	n.d.
Tillandsia tenuifolia L.	B0026	5	2	1177	10	0	1	2	1112	1112	1112	2n = 2x
Tillandsia aff. *tenuifolia* L.	B0414	0	1	1177	2	0	1	2	1115	1115	1115	2n = 2x
Tillandsia subg. ***Diaphoranthema*** (Beer) Baker												
Tillandsia capillaris Ruiz & Pav.	B0518	11	4	1180	13	2	1	2	1054	?	?	2n = 4x!
Tillandsia funebris A. Cast.	B0089	1	2	1177	9	3	1	2	1141	1141	?	2n = 2x
Tillandsia kuehhasii W. Till	B0396	1	2	1180	7	1	1	2	1053	1053	1051	2n = 2x
Tillandsia landbeckii subsp. *andina* W. Till	B0423	0	1	1180	0	0	1	1	1131	1131	1131	2n = 2x
Tillandsia mollis H. Hrom. & W. Till	B0206	4	2	1177	8	2	1	2	1133	?	?	2n = 2x
Tillandsia recurvata (L.) L.	B0529	0	1	1177	0	0	0	1	1145	1145	1145	2n = 2x
Tillandsia recurvata (L.) L.	B0540	0	1	1177	0	0	1	1	1145	1145	1145	2n = 2x
Tillandsia usneoides (L.) L.	B0083	4	2	1177	3	1	1	2	1130	1130	1129	2n = 2x
Tillandsia usneoides (L.) L.	B0109	3	2	1177	8	1	1	2	1133	1134	1133	2n = 2x
Tillandsia usneoides (L.) L.	B0451	3	2	1177	9	1	1	2	1133	1133	1132	2n = 2x
Tillandsia cf. *usneoides* (L.) L.	B0539	0	1	1177	0	0	1	1	1128	1128	1128	2n = 2x
Tillandsia virescens Ruiz & Pav.	B0516	3	4	1180	9	1	1	2	1056	1056	1054	2n = 4x!
Tillandsia subg. ***Phytarrhiza*** (Vis.) Baker												
Tillandsia duratii Vis.	B0088	5	2	1180	7	0	0	2	1134	1134	1134	2n = 2x
Tillandsia duratii Vis.	B0737	4	2	1180	8	0	1	2	1134	1134	1134	2n = 2x
Tillandsia duratii Vis.	B0740	5	2	1180	9	1	1	2	1135	1135	1134	2n = 2x
Tillandsia kirschnekii Rauh & W. Till	B0384	1	2	1180	4	1	1	2	1134	1134	1133	2n = 2x
Tillandsia paleacea C. Presl	B0404	4	2	1180	11	1	1	2	1137	1137	1134	2n = 2x
Tillandsia aff. *streptocarpa* Baker	B0226	2	2	1180	3	1	1	2	1135	1135	1134	2n = 2x
Tillandsia subg. ***Pseudalcantarea*** Mez												
Tillandsia grandis Schltdl.	B0124	2	2	1177	2	0	1	2	1141	1141	1141	2n = 2x
Tillandsia grandis Schltdl.	B0125	1	2	1177	9	2	1	2	1148	1148	1147	2n = 2x
Tillandsia macropetala Wawra	B0742	2	2	1177	3	3	0	2	1142	1142	1136	2n = 2x
Tillandsia macropetala Wawra	B0748	2	2	1177	1	1	1	2	1139	1139	1136	2n = 2x
Tillandsia viridiflora (Beer) Baker	B0006	0	1	1177	0	1	1	2	1139	1139	1138	2n = 2x
Tillandsia subg. ***Pseudovriesea*** Barfuss & W. Till												
Tillandsia andreettae (Rauh) J.R. Grant	B0373	0	1	1177	0	0	0	1	1136	1136	1136	2n = 2x
Tillandsia appenii (Rauh) J.R. Grant	B0066	0	1	1177	4	0	1	2	1133	1133	1133	2n = 2x
Tillandsia appenii (Rauh) J.R. Grant	B0464	3	2	1177	1	1	1	2	1115	1115	1114	2n = 2x
Tillandsia arpocalyx André	B0552	0	1	1177	1	1	1	2	1176	1176	1132	2n = 2x
Tillandsia barclayana Baker	B0028	n.d.	n.d.	n.d.	n.d.	n.d	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Tillandsia barclayana Baker	B0142	3	2	1177	4	2	1	2	1137	1137	1134	2n = 2x

		PHYC			*PRK*							
Taxon	**DNA no.**	**SNPs**	**Geno types**	**Length**	**SNPs**	**Indels**	**SSR**	**Geno types**	**Consensus length**	**Allele 1 length (a1)**	**Allele 2 length (a2)**	**postulated ploidy level**
Tillandsia barthlottii Rauh	B0035	3	2	1177	0	1	1	2	1133	1133	1130	2n = 2x
Tillandsia barthlottii Rauh	B0716	1	2	1177	3	1	1	2	1134	1134	1133	2n = 2x
Tillandsia cereicola Mez	B0134	1	2	1177	7	1	1	2	1137	1138	1137	2n = 2x
Tillandsia cereicola Mez	B0476	0	1	1177	2	1	1	2	1142	1142	1136	2n = 2x
Tillandsia espinosae L.B. Sm.	B0143	0	1	1177	0	0	0	1	1135	1135	1135	2n = 2x
Tillandsia espinosae L.B. Sm.	B0462	0	1	1177	2	0	1	2	1136	1136	1136	2n = 2x
Tillandsia fragrans André	B0632	2	2	1177	10	1	0	2	1132	1132	1131	2n = 2x
Tillandsia frank-hasei J.R. Grant	B0315	0	1	1177	0	0	1	1	1131	1131	1131	2n = 2x
Tillandsia hitchcockiana L.B. Sm.	B0468	0	1	1177	2	0	0	2	1137	1137	1137	2n = 2x
Tillandsia hitchcockiana L.B. Sm.	B0629	2	2	1177	0	0	0	1	1137	1137	1137	2n = 2x
Tillandsia myriantha Baker	B0760	0	1	1177	0	0	0	1	1102	1102	1102	2n = 2x
Tillandsia peruviana J.R. Grant	B0470	0	1	1177	2	0	1	2	1136	1136	1136	2n = 2x
Tillandsia petraea L.B. Sm.	B0630	1	2	1177	0	0	0	1	1110	1110	1110	2n = 2x
Tillandsia cf. *porphyrocraspeda* J.R. Grant	B0350	0	1	1177	3	2	1	2	1132	1132	1132	2n = 2x
Tillandsia spathacea Mez & Sodiro	B0565	0	1	1177	0	0	0	1	1130	1130	1130	2n = 2x
Tillandsia strobeliae (Rauh) J.R. Grant	B0198	2	2	1177	4	1	1	2	1131	1131	1130	2n = 2x
Tillandsia tequendamae André	B0569	0	1	1177	0	0	0	1	1131	1131	1131	2n = 2x
Tillandsia werneriana J.R. Grant	B0067	0	1	1177	2	2	1	2	1137	1137	1135	2n = 2x
Tillandsia cf. *werneriana* J.R. Grant	B0144	2	2	1177	2	1	0	2	1149	1149	1148	2n = 2x
Tillandsia cf. *werneriana* J.R. Grant	B0514	3	2	1177	7	0	0	2	1136	1136	1136	2n = 2x
Tillandsia subg. ***Tillandsia***												
Tillandsia achyrostachys E. Morren ex Baker	B0408	6	2	1177	4	1	0	2	1103	1103	1099	2n = 2x
Tillandsia adpressiflora Mez	B0597	2	2	1180	0	0	1	1	1139	1139	1139	2n = 2x
Tillandsia andrieuxii (Mez) L.B. Sm.	B0063	1	2	1177	6	1	0	2	1098	1098	1080	2n = 2x
Tillandsia baileyi Rose ex Small	B0421	5	2	1180	8	1	0	2	1140	1140	1139	2n = 2x
Tillandsia cf. *beutelspacheri* Matuda	B0360	2	2	1177	9	2	0	2	1138	1138	1137	2n = 2x
Tillandsia caput-medusae E. Morren	B0046	0	1	1180	0	0	0	1	1142	1142	1142	2n = 2x
Tillandsia aff. *caput-medusae* E. Morren	B0424	5	2	1180	1	0	2	2	1162	1162	1162	2n = 2x
Tillandsia cryptopoda L.B. Sm.	B0787	0	1	1177	0	0	1	1	1133	1133	1133	2n = 2x
Tillandsia aff. *cucaensis* Wittm.	B0732	15	2	1177	0	0	0	1	1135	1135	1135	2n = 2x
Tillandsia durangensis Rauh & Ehlers	B0365	6	2	1180	0	0	1	1	1140	1140	1140	2n = 2x
Tillandsia ehlersiana Rauh	B0431	1	2	1180	2	0	1	2	1140	1140	1140	2n = 2x
Tillandsia exserta Fernald	B0390	1	2	1177	5	0	0	2	1135	1135	1135	2n = 2x
Tillandsia extensa Mez	B0712	1	2	1177	9	1	1	2	1130	1131	1130	2n = 2x
Tillandsia fasciculata Sw.	B0076	2	2	1177	18	3	0	2	1136	1131!	1134!	2n = 2x
Tillandsia filifolia Schltdl. & Cham.	B0788	8	2	1177	9	3	1	2	1121	1121	1120	2n = 2x
Tillandsia filifolia Schltdl. & Cham.	B0790	5	2	1177	12	2	1	2	1123	1123	1121	2n = 2x
Tillandsia fuchsii W. Till	B0391	3	2	1177	11	1	0	2	1120	1120	1118	2n = 2x
Tillandsia glabrior (L.B. Sm.) López-Ferr., Espejo & I. Ramírez	B0340	5	2	1180	2	2	0	2	1138	1138	1137	2n = 2x
Tillandsia guatemalensis L.B. Sm.	B0008	4	2	1177	11	1	0	2	1129	1129	1128	2n = 2x
Tillandsia guatemalensis L.B. Sm.	B0103	3	2	1177	7	0	0	2	1128	1128	1128	2n = 2x
Tillandsia guatemalensis L.B. Sm.	B0104	8	2	1177	1	0	0	2	1129	1129	1129	2n = 2x
Tillandsia guerreroensis Rauh	B0349	6	2	1177	8	3	1	2	1137	?	?	2n = 2x
Tillandsia hildae Rauh	B0763	2	2	1180	7	1	0	2	1130	1130	1116	2n = 2x
Tillandsia huajuapanensis Ehlers & Lautner	B0713	3	2	1177	6	1	0	2	1130	1130	1129	2n = 2x
Tillandsia ionantha Planch.	B0084	3	2	1180	7	0	2	2	1136	1136	1136	2n = 2x

		PHYC			*PRK*							
Taxon	**DNA no.**	**SNPs**	**Geno types**	**Length**	**SNPs**	**Indels**	**SSR**	**Geno types**	**Consensus length**	**Allele 1 length (a1)**	**Allele 2 length (a2)**	**postulated ploidy level**
Tillandsia juerg-rutschmannii Rauh	B0715	1	2	1177	4	1	0	2	1137	1137	1136	2n = 2x
Tillandsia leiboldiana Schltdl.	B0323	4	2	1177	0	1	0	2	1116	1116	1106	2n = 2x
Tillandsia malzinei (E. Morren) Baker	B0145	0	1	1177	0	0	0	1	1125	1125	1125	2n = 2x
Tillandsia malzinei (E. Morren) Baker	B0492	0	1	1177	3	1	0	2	1125	1125	1110	2n = 2x
Tillandsia mima var. *chiletensis* Rauh	B0708	2	2	1177	1	0	1	2	1130	1130	1130	2n = 2x
Tillandsia mima var. *chiletensis* Rauh	B0710	2	2	1177	1	0	1	2	1130	1130	1130	2n = 2x
Tillandsia cf. *mima* L.B. Sm. var. *mima*	B0709	5	2	1177	0	0	0	1	1130	1130	1130	2n = 2x
Tillandsia mitlaensis var. *tulensis* Lautner & Ehlers	B0402	0	1	1177	8	0	1	2	1141	1141	1141	2n = 2x
Tillandsia moronesensis Ehlers	B0380	0	1	1177	1	0	0	2	1137	1137	1137	2n = 2x
Tillandsia paniculata (L.) L.	B0102	0	1	1177	0	0	1	1	1130	1130	1130	2n = 2x
Tillandsia plagiotropica Rohweder	B0386	0	1	1177	0	0	0	1	1116	1116	1116	2n = 2x
Tillandsia praschekii Ehlers & Willinger	B0364	0	1	1177	3	2	1	2	1140	1142	1140	2n = 2x
Tillandsia propagulifera Rauh	B0310	2	2	1177	8	2	1	2	1131	1130	1130	2n = 2x
Tillandsia pseudosetacea Ehlers & Rauh	B0333	0	1	1177	0	0	0	1	1135	1135	1135	2n = 2x
Tillandsia pueblensis L.B. Sm.	B0398	2	2	1180	8	2	1	2	1144	1146	1144	2n = 2x
Tillandsia punctulata Schltdl.& Cham.	B0061	0	1	1177	0	0	0	1	1138	1138	1138	2n = 2x
Tillandsia remota Wittm.	B0072	4	2	1177	4	0	0	2	1135	1135	1135	2n = 2x
Tillandsia cf. *rhomboidea* André	B0306	0	1	1177	0	0	0	1	1134	1134	1134	2n = 2x
Tillandsia cf. *rhomboidea* André	B0372	0	1	1177	0	0	1	1	1134	1134	1134	2n = 2x
Tillandsia schatzlii Rauh	B0416	0	1	1177	0	0	1	1	1144	1144	1144	2n = 2x
Tillandsia secunda Kunth	B0527	0	1	1177	3	0	1	2	1129	1129	1129	2n = 2x
Tillandsia secunda Kunth	B0528	0	1	1177	3	1	1	2	1130	1129	1128	2n = 2x
Tillandsia secunda Kunth	B0549	3	2	1177	2	1	1	2	1130	1130	1129	2n = 2x
Tillandsia secunda Kunth	B0551	3	2	1177	3	0	1	2	1129	1129	1129	2n = 2x
Tillandsia seleriana Mez	B0413	5	2	1180	0	0	1	1	1144	1144	1144	2n = 2x
Tillandsia selleana Harms	B0243	0	1	1177	0	0	0	1	1128	1128	1128	2n = 2x
Tillandsia spiraliflora Rauh	B0762	4	2	1177	8	0	1	2	1130	1130	1130	2n = 2x
Tillandsia tricolor Schltdl. & Cham.	B0785	3	2	1180	4	1	0	2	1131	1131	1114	2n = 2x
Tillandsia utriculata L.	B0100	0	1	1180	0	0	1	1	1143	1143	1143	2n = 2x
Tillandsia utriculata L.	B0805	0	1	1180	0	0	1	1	1143	1143	1143	2n = 2x
Tillandsia utriculata L.	B0807	0	1	1180	0	0	1	1	1140	1140	1140	2n = 2x
Tillandsia aff. *utriculata* L.	B0743	0	1	1180	0	0	1	1	1143	1143	1143	2n = 2x
Tillandsia aff. *utriculata* L.	B0747	0	1	1180	0	0	1	1	1143	1143	1143	2n = 2x
Tillandsia variabilis Schltdl.	B0308	0	1	1180	0	0	0	1	1129	1129	1129	2n = 2x
Tillandsia velutina Ehlers	B0427	0	1	1177	4	2	0	2	1123	?	?	2n = 2x
Tillandsia subg. ***Viridantha*** (Espejo) Barfuss & W. Till												
Tillandsia atroviridipetala Matuda	B0215	0	1	1177	3	0	1	2	1082	1082	1082	2n = 2x
Tillandsia balsasensis Rauh	B0221	0	1	1177	0	0	1	1	1127	1127	1127	2n = 2x
Tillandsia chusgonensis L. Hrom.	B0210	5	2	1177	5	1	1	2	1085	1085	1084	2n = 2x
Tillandsia heteromorpha Mez	B0224	0	1	1177	0	0	0	1	1081	1081	1081	2n = 2x
Tillandsia ignesiae Mez	B0222	7	2	1177	7	1	1	2	1084	1084	1081	2n = 2x
Tillandsia lepidosepala L.B. Sm.	B0219	1	2	1177	0	0	1	1	1083	1083	1083	2n = 2x
Tillandsia mauryana L.B. Sm.	B0238	3	2	1177	2	2	1	2	1084	1084	1082	2n = 2x
Tillandsia oblivata L. Hrom.	B0205	2	2	1177	0	1	1	2	1081	1081	1067	2n = 2x
Tillandsia plumosa Baker	B0086	2	2	1177	2	1	1	2	1082	1082	1081	2n = 2x
Tillandsia reducta L.B. Sm.	B0209	0	1	1177	4	1	1	2	1084	1084	1080	2n = 2x

		PHYC			*PRK*							
Taxon	**DNA no.**	**SNPs**	**Geno types**	**Length**	**SNPs**	**Indels**	**SSR**	**Geno types**	**Consensus length**	**Allele 1 length (a1)**	**Allele 2 length (a2)**	**postulated ploidy level**
Tillandsia rupicola Baker	B0039	0	1	1177	0	0	0	1	1096	1096	1096	2n = 2x
Tillandsia stellifera L. Hrom.	B0239	0	1	1177	0	0	0	1	1081	1081	1081	2n = 2x
Tillandsia tectorum var. *globosa* L. Hrom.	B0248	0	1	1177	2	0	0	2	1094	1094	1094	2n = 2x
Tillandsia tectorum var. *tectorum* f. *gigantea* L. Hrom.	B0253	4	2	1177	2	0	0	2	1097	1097	1097	2n = 2x
Tillandsia tectorum E. Morren var. *tectorum* f. *tectorum*	B0249	0	1	1177	4	3	0	2	1131	1131	1127	2n = 2x
Tillandsia tectorum var. *viridula* L. Hrom.	B0245	0	1	1177	1	0	1	2	1127	1127	1127	2n = 2x
Tillandsia tomekii L. Hrom.	B0218	1	2	1177	6	1	1	2	1128	1128	1127	2n = 2x
Tillandsia tortilis Klotzsch ex Baker	B0049	2	2	1177	1	0	1	2	1087	1087	1087	2n = 2x
Biflora clade												
Tillandsia baliophylla Harms	B0101	0	1	1177	0	0	1	1	1108	1108	1108	2n = 2x
Tillandsia biflora Ruiz & Pav.	B0090	0	1	1177	0	0	1	1	1641	1641	1641	2n = 2x
Tillandsia brenneri Rauh	B0236	0	1	1180	0	0	1	1	1122	1122	1122	2n = 2x
Tillandsia brevilingua Mez ex Harms	B0056	0	1	1180	0	1	0	2	1124	1124	1123	2n = 2x
Tillandsia brevilingua Mez ex Harms	B0252	1	2	1180	0	1	0	2	1124	1124	1123	2n = 2x
Tillandsia buseri var. *nubicola* Gilmartin	B0561	0	1	1177	1	0	1	2	1101	1101	1101	2n = 2x
Tillandsia cf. *buseri* var. *nubicola* Gilmartin	B0441	1	2	1177	0	0	1	1	1124	1124	1124	2n = 2x
Tillandsia cf. *buseri* var. *nubicola* Gilmartin	B0442	4	2	1177	1	0	1	2	1117	1117	1117	2n = 2x
Tillandsia complanata Benth.	B0244	4	2	1177	1	1	0	2	1131	1131	1129	2n = 2x
Tillandsia complanata Benth.	B0562	1	2	1177	8	1	0	2	1133	1133	1129	2n = 2x
Tillandsia complanata Benth.	B0563	3	2	1177	6	1	0	2	1129	1129	1128	2n = 2x
Tillandsia confertiflora André	B0614	4	2	1177	0	0	1	1	1119	1119	1119	2n = 2x
Tillandsia confinis L.B. Sm.	B0587	2	2	1177	0	2	1	2	1134	1134	1127	2n = 2x
Tillandsia demissa L.B. Sm.	B0075	3	2	1177	0	0	0	1	1124	1124	1124	2n = 2x
Tillandsia denudata var. *vivipara* Rauh	B0750	2	2	1177	1	0	0	2	1135	1135	1135	2n = 2x
Tillandsia deppeana Steud.	B0457	0	1	1177	3	0	0	2	1111	1111	1111	2n = 2x
Tillandsia deppeana Steud.	B0458	0	1	1177	2	1	0	2	1111	1111	1109	2n = 2x
Tillandsia fendleri Griseb.	B0009	0	1	1177	0	2	0	2	1116	1116	1114	2n = 2x
Tillandsia fendleri Griseb.	B0445	0	1	1177	0	0	1	1	1117	1117	1117	2n = 2x
Tillandsia floribunda Kunth	B0351	1	2	1180	4	1	1	2	1068	1068	1067	2n = 2x
Tillandsia fosteri Gilmartin	B0639	3	2	1177	12	2	1	2	1123	1123	1117	2n = 2x
Tillandsia glauca L.B. Sm.	B0618	1	2	1177	0	0	1	1	1117	1117	1117	2n = 2x
Tillandsia heterophylla E. Morren	B0047	0	1	1177	0	0	0	1	1110	1110	1110	2n = 2x
Tillandsia imperialis E. Morren ex Roezl	B0292	1	2	1177	8	1	1	2	1113	1113	1111	2n = 2x
Tillandsia incarnata Kunth	B0522	0	1	1177	0	0	1	1	1113	1113	1113	2n = 2x
Tillandsia ionochroma André ex Mez	B0600	0	1	1177	0	0	1	1	1692	1692	1692	2n = 2x
Tillandsia kauffmannii Ehlers	B0074	0	1	1177	0	0	1	1	1114	1114	1114	2n = 2x
Tillandsia krahnii Rauh	B0235	0	1	1177	0	0	0	1	1120	1120	1120	2n = 2x
Tillandsia lajensis André	B0242	0	1	1177	1	0	1	2	1120	1120	1120	2n = 2x
Tillandsia lajensis André	B0546	0	1	1177	0	0	1	1	1120	1120	1120	2n = 2x
Tillandsia lajensis André	B0554	0	1	1177	1	0	1	2	1120	1120	1120	2n = 2x
Tillandsia lajensis André	B0555	0	1	1177	0	0	1	1	1120	1120	1120	2n = 2x
Tillandsia latifolia var. *divaricata* (Benth.) Mez	B0068	2	2	1177	7	2	0	2	1115	1116!	1100!	2n = 2x
Tillandsia latifolia Meyen var. *latifolia*	B0433	3	2	1177	4	1	1	2	1115	1115	1098	2n = 2x
Tillandsia latifolia var. *leucophylla* Rauh	B0228	1	2	1177	6	1	1	2	1115	1115	1098	2n = 2x
Tillandsia macbrideana L.B. Sm.	B0070	2	2	1177	13	2	1	2	1113	?	?	2n = 2x
Tillandsia maculata Ruiz & Pav.	B0574	4	2	1177	1	1	0	2	1124	1124	1116	2n = 2x

		PHYC			*PRK*							
Taxon	**DNA no.**	**SNPs**	**Geno types**	**Length**	**SNPs**	**Indels**	**SSR**	**Geno types**	**Consensus length**	**Allele 1 length (a1)**	**Allele 2 length (a2)**	**postulated ploidy level**
Tillandsia multicaulis Steud.	B0107	0	1	1177	0	0	0	1	1115	1115	1115	2n = 2x
Tillandsia orbicularis L.B. Sm.	B0375	0	1	1177	0	0	1	1	1085	1085	1085	2n = 2x
Tillandsia pastensis André	B0521	0	1	1177	0	0	0	1	1095	1095	1095	2n = 2x
Tillandsia pseudomacbrideana Rauh	B0036	3	2	1177	8	1	1	2	1129	1129	1116	2n = 2x
Tillandsia roezlii E. Morren	B0764	1	2	1177	1	0	1	2	1115	1115	1115	2n = 2x
Tillandsia rudolfii E. Gross & Hase	B0654	0	1	1177	7	2	1	2	1113	1113	1113	2n = 2x
Tillandsia somnians L.B. Sm.	B0301	1	2	1177	8	0	1	2	1100	1100	1100	2n = 2x
Tillandsia somnians L.B. Sm.	B0312	0	1	1177	0	0	1	1	1100	1100	1100	2n = 2x
Tillandsia stenoura Harms	B0635	0	1	1177	10	2	1	2	1114	1114	1109	2n = 2x
Tillandsia subconcolor L.B. Sm.	B0227	0	1	1177	0	0	1	1	1115	1115	1115	2n = 2x
Tillandsia cf. *superba* Mez & Sodiro	B0602	3	2	1177	0	0	1	1	1101	1101	1101	2n = 2x
Tillandsia tovarensis Mez	B0605	1	2	1177	0	0	0	1	1104	1104	1104	2n = 2x
Tillandsia turneri Baker	B0650	0	1	1177	0	0	1	1	1081	1081	1081	2n = 2x
Tillandsia sp.	B0634	6	2	1177	8	0	1	2	1088	1088	1088	2n = 2x
Gardneri clade												
Tillandsia brachyphylla Baker	B0082	2	2	1177	2	0	1	2	1097	1097	1097	2n = 2x
Tillandsia gardneri Lindl.	B0041	0	1	1177	0	0	1	1	1092	1092	1092	2n = 2x
Tillandsia globosa Wawra	B0419	5	2	1177	10	2	0	2	1084	?	?	2n = 2x
Purpurea clade												
Tillandsia aurea Mez	B0250	0	1	1177	6	0	0	2	1096	1096	1096	2n = 2x
Tillandsia cacticola L.B. Sm.	B0044	0	1	1177	1	0	0	2	1096	1096	1096	2n = 2x
Tillandsia purpurea Ruiz & Pav.	B0246	0	1	1177	0	0	0	1	1095	1095	1095	2n = 2x
Tillandsia straminea Kunth	B0247	3	2	1177	0	0	0	1	1096	1096	1096	2n = 2x
Rauhii clade												
Tillandsia ecarinata L.B. Sm.	B0237	1	2	1180	5	1	1	2	1134	1134	1132	2n = 2x
Tillandsia ferreyrae L.B. Sm.	B0241	1	2	1177	2	2	1	2	1132	1132	1130	2n = 2x
Tillandsia rauhii L.B. Sm.	B0092	0	1	1177	0	0	0	1	1128	1128	1128	2n = 2x
Tillandsia teres L.B. Sm.	B0201	3	2	1177	8	0	0	2	1128	1128	1128	2n = 2x
Xiphioides clade												
Tillandsia aff. *arequitae* (André) André ex Mez	B0387	0	1	1177	0	0	1	1	1132	1132	1132	2n = 2x
Tillandsia argentina C.H. Wright	B0087	2	2	1177	9	3	1	2	1129	1127!	1125!	2n = 2x
Tillandsia argentina C.H. Wright	B0797	3	2	1177	4	0	1	2	1129	1129	1129	2n = 2x
Tillandsia bermejoensis H. Hrom.	B0034	1	2	1177	0	0	1	1	1130	1130	1130	2n = 2x
Tillandsia buchlohii Rauh	B0395	5	2	1177	4	0	1	2	1129	1129	1129	2n = 2x
Tillandsia cardenasii L.B. Sm.	B0409	0	1	1177	4	1	1	2	1138	1138	1136	2n = 2x
Tillandsia caulescens Brongn. ex Baker	B0071	0	1	1177	0	0	1	1	1130	1130	1130	2n = 2x
Tillandsia cochabambae E. Gross & Rauh	B0392	6	2	1177	8	1	1	2	1132	1132	1131	2n = 2x
Tillandsia comarapaensis H. Luther	B0407	5	2	1177	2	0	1	2	1129	1129	1129	2n = 2x
Tillandsia diaguitensis A. Cast.	B0429	7	2	1177	8	0	1	2	1129	1129	1129	2n = 2x
Tillandsia didisticha (E. Morren) Baker	B0038	1	2	1177	0	0	1	1	1130	1130	1130	2n = 2x
Tillandsia gerdae Ehlers	B0411	6	2	1177	8	3	1	2	1131	?	?	2n = 2x
Tillandsia koehresiana Ehlers	B0371	1	2	1177	5	2	1	2	1131	1131	1127	2n = 2x
Tillandsia lorentziana Griseb.	B0374	0	1	1177	4	3	1	2	1139	?	?	2n = 2x
Tillandsia aff. *lotteae* H. Hrom.	B0808	0	1	1177	0	0	1	1	1131	1131	1131	2n = 2x
Tillandsia aff. *lotteae* H. Hrom.	B0809	0	1	1177	0	0	1	1	1131	1131	1131	2n = 2x
Tillandsia markusii L. Hrom.	B0399	1	2	1177	7	3	1	2	1129	1129	1126	2n = 2x

		PHYC			*PRK*							
Taxon	**DNA no.**	**SNPs**	**Geno types**	**Length**	**SNPs**	**Indels**	**SSR**	**Geno types**	**Consensus length**	**Allele 1 length (a1)**	**Allele 2 length (a2)**	**postulated ploidy level**
Tillandsia muhriae W. Weber	B0438	0	1	1177	6	0	1	2	1130	1130	1130	2n = 2x
Tillandsia ramellae W. Till & S. Till	B0430	0	1	1177	0	2	1	2	1138	?	?	2n = 2x
Tillandsia rosacea L. Hrom. & W. Till	B0405	4	2	1177	1	0	1	2	1128	1128	1128	2n = 2x
Tillandsia rosarioae L. Hrom.	B0403	5	2	1177	5	2	1	2	1129	1129	1127	2n = 2x
Tillandsia aff. *vernicosa* Baker	B0355	0	1	1177	11	0	1	2	1129	1129	1129	2n = 2x
Tillandsia xiphioides subsp. *prolata* H. Luther	B0418	1	2	1177	6	2	1	2	1130	?	?	2n = 2x
Tillandsia xiphioides Ker Gawl. subsp. *xiphioides*	B0040	3	2	1177	8	1	1	2	1130	1130	1129	2n = 2x
Tillandsia yuncharaensis W. Till	B0417	0	1	1177	7	0	1	2	1129	1129	1129	2n = 2x
Tillandsia zecheri var. *cafayatensis* Palací & G.K. Br.	B0361	5	2	1177	11	3	1	2	1138	?	?	2n = 2x
Incertae sedis												
Tillandsia albertiana F. Verv.	B0033	2	2	1177	0	0	1	1	1138	1138	1138	2n = 2x
Tillandsia albertiana F. Verv.	B0212	0	1	1177	0	0	1	1	1136	1136	1136	2n = 2x
Tillandsia albertiana F. Verv.	B0225	0	1	1177	4	0	1	2	1141	1141	1141	2n = 2x
Tillandsia australis Mez	B0203	0	1	1177	0	0	1	1	1115	1115	1115	2n = 2x
Tillandsia australis Mez	B0759	0	1	1177	0	0	1	1	1115	1115	1115	2n = 2x
Tillandsia disticha Kunth	B0048	0	1	1177	2	1	1	2	1131	1131	1130	2n = 2x
Tillandsia disticha Kunth	B0202	3	2	1177	1	1	1	2	1130	1131	1130	2n = 2x
Tillandsia disticha Kunth	B0233	2	2	1177	0	0	1	1	1134	1134	1134	2n = 2x
Tillandsia edithae Rauh	B0425	2	2	1177	2	0	1	2	1136	1136	1136	2n = 2x
Tillandsia edithae Rauh	B0436	0	1	1177	0	0	1	1	1136	1136	1136	2n = 2x
Tillandsia esseriana Rauh & L.B. Sm.	B0069	0	1	1177	7	2	1	2	1157	1157!	1128!	2n = 2x
Tillandsia esseriana Rauh & L.B. Sm.	B0200	3	2	1177	0	0	1	1	1129	1129	1129	2n = 2x
Tillandsia nana Baker	B0343	0	1	1177	1	1	1	2	1133	1133	1132	2n = 2x
Tillandsia pseudomicans Rauh	B0347	3	2	1177	3	0	1	2	1115	1115	1115	2n = 2x
Tillandsia sphaerocephala Baker	B0366	1	2	1177	2	1	0	2	1135	1135	1134	2n = 2x
Bromeliaceae tribe ***Vrieseeae*** W. Till & Barfuss												
Bromeliaceae subtribe ***Cipuropsidinae*** Barfuss & W. Till												
Cipuropsis Ule												
Cipuropsis amicorum (I. Ramírez & Bevil.) Barfuss & W. Till	B0318	1	2	1177	0	0	0	1	903	903	903	2n = 2x
Cipuropsis zamorensis (L.B. Sm.) Barfuss & W. Till	B0045	0	1	1177	4	0	0	2	915	915	915	2n = 2x
Cipuropsis cv. Elan	B0117	5	2	1177	7	1	0	2	918	918	915	2n = 2x
Mezobromelia L.B. Sm.												
Mezobromelia bicolor L.B. Sm.	B0578	3	2	1177	3	2	0	2	902	?	?	2n = 2x
Mezobromelia bicolor L.B. Sm.	B0583	1	2	1177	6	0	0	2	901	901	901	2n = 2x
Mezobromelia pleiosticha (Griseb.) Utley & H. Luther	B0274	0	1	1177	0	0	0	1	923	923	923	2n = 2x
Mezobromelia pleiosticha (Griseb.) Utley & H. Luther	B0649	0	1	1177	0	0	0	1	923	923	923	2n = 2x
Mezobromelia cf. *pleiosticha* (Griseb.) Utley & H. Luther	B0330	5	2	1177	5	1	0	2	901	901	899	2n = 2x
Mezobromelia schimperiana (Wittm.) Barfuss & Manzan.	B0334	2	2	1177	4	1	0	2	901	901	899	2n = 2x
Mezobromelia schimperiana (Wittm.) Barfuss & Manzan.	B0589	1	2	1177	8	0	0	2	899	899	899	2n = 2x
Mezobromelia schimperiana (Wittm.) Barfuss & Manzan.	B0611	2	2	1177	3	1	0	2	901	901	899	2n = 2x
Mezobromelia schimperiana (Wittm.) Barfuss & Manzan.	B0612	0	1	1177	6	1	0	2	901	901	899	2n = 2x
Mezobromelia aff. *schimperiana* (Wittm.) Barfuss & Manzan.	B0197	0	1	1177	0	0	0	1	886	886	886	2n = 2x
Mezobromelia sp.	B0194	1	2	1177	5	0	0	2	901	901	901	2n = 2x
Werauhia J.R. Grant												
Werauhia ampla (L.B. Sm.) J.R. Grant	B0132	4	2	1177	7	0	0	2	1015	1015	1015	2n = 2x

		PHYC			*PRK*							
Taxon	**DNA no.**	**SNPs**	**Geno types**	**Length**	**SNPs**	**Indels**	**SSR**	**Geno types**	**Consensus length**	**Allele 1 length (a1)**	**Allele 2 length (a2)**	**postulated ploidy level**
Werauhia gladioliflora (H. Wendl.) J.R. Grant	B0149	0	1	1177	0	0	0	1	1026	1026	1026	2n = 2x
Werauhia gladioliflora (H. Wendl.) J.R. Grant	B0509	0	1	1177	0	0	0	1	1026	1026	1026	2n = 2x
Werauhia guadelupensis (Baker) J.R. Grant	B0313	0	1	1177	0	0	0	1	1079	1079	1079	2n = 2x
Werauhia haltonii (H. Luther) J.R. Grant	B0580	0	1	1180	0	0	0	1	975	975	975	2n = 2x
Werauhia hygrometrica (André) J.R. Grant	B0598	0	1	1180	0	0	0	1	975	975	975	2n = 2x
Werauhia hygrometrica (André) J.R. Grant	B0621	0	1	1180	0	0	0	1	975	975	975	2n = 2x
Werauhia insignis (Mez) W. Till, Barfuss & R. Samuel	B0017	0	1	1159	0	0	0	1	1031	1031	1031	2n = 2x
Werauhia ororiensis (Mez) J.R. Grant	B0025	2	2	1180	9	2	0	2	962	962	954	2n = 2x
Werauhia pedicellata (Mez & Werckle) J.R. Grant	B0752	0	1	1180	0	0	0	1	1016	1016	1016	2n = 2x
Werauhia ringens (Griseb.) J.R. Grant	B0019	0	1	1177	0	0	0	1	1045	1045	1045	2n = 2x
Werauhia sintenisii (Baker) J.R. Grant	B0450	0	1	1180	0	0	0	1	1031	1031	1031	2n = 2x
Werauhia tarmaensis (Rauh) J.R. Grant	B0018	0	1	1177	0	0	0	1	1081	1081	1081	2n = 2x
Werauhia viridiflora (Regel) J.R. Grant	B0285	0	1	1177	0	0	0	1	908	908	908	2n = 2x
Werauhia viridiflora (Regel) J.R. Grant	B0325	7	2	1177	3	0	0	2	908	908	908	2n = 2x
Werauhia werckleana (Mez) J.R. Grant	B0497	3	2	1177	8	0	0	2	1083	1083	1083	2n = 2x
Chrysostachys clade												
Vriesea chrysostachys E. Morren	B0005	0	1	1177	0	0	0	1	1029	1029	1029	2n = 2x
Vriesea ospinae H. Luther	B0054	0	1	1177	4	1	0	2	1036	1036	1035	2n = 2x
Singularis clade												
Tillandsia asplundii L.B. Sm.	B0588	0	1	1177	2	2	0	2	921	921	918	2n = 2x
Tillandsia singularis Mez & Wercklé	B0064	0	1	1180	0	0	0	1	914	914	914	2n = 2x
Splendens clade												
Vriesea glutinosa Lindl.	B0130	0	1	1177	0	0	0	1	1067	1067	1067	2n = 2x
Vriesea splendens (Brongn.) Lem.	B0037	0	1	1177	0	0	1	1	1069	1069	1069	2n = 2x
Incertae sedis												
Vriesea tuerckheimii (Mez) L.B. Sm.	B0148	0	1	1177	1	0	0	2	1090	1090	1090	2n = 2x
Bromeliaceae subtribe ***Vrieseinae*** W. Till & Barfuss												
Alcantarea (E. Morren ex Mez) Harms												
Alcantarea benzingii Leme	B0166	0	1	1177	0	0	0	1	1125	1125	1125	2n = 2x
Alcantarea burle-marxii (Leme) J.R. Grant	B0159	2	2	1177	11	1	1	2	1134	1134	1133	2n = 2x
Alcantarea duarteana (L.B. Sm.) J.R. Grant	B0059	0	1	1177	4	0	0	2	1133	1133	1133	2n = 2x
Alcantarea duarteana (L.B. Sm.) J.R. Grant	B0165	0	1	1177	3	1	0	2	1133	1133	1132	2n = 2x
Alcantarea edmundoi (Leme) J.R. Grant	B0161	2	2	1177	1	0	1	2	1134	1134	1134	2n = 2x
Alcantarea edmundoi (Leme) J.R. Grant	B0171	0	1	1177	0	0	0	1	1135	1135	1135	2n = 2x
Alcantarea aff. *extensa* (L.B. Sm.) J.R. Grant	B0164	4	2	1177	7	1	1	2	1138	1138	1133	2n = 2x
Alcantarea farneyi (Martinelli & A.F. Costa) J.R. Grant	B0170	0	1	1177	4	0	1	2	1135	1135	1135	2n = 2x
Alcantarea geniculata (Wawra) J.R. Grant	B0153	2	2	1177	0	0	0	1	1133	1133	1133	2n = 2x
Alcantarea cf. *geniculata* (Wawra) J.R. Grant	B0751	0	1	1177	0	0	1	1	1134	1134	1134	2n = 2x
Alcantarea glazioviana (Lem.) Leme	B0167	0	1	1177	10	2	0	2	1134	?	?	2n = 2x
Alcantarea heloisae J.R. Grant	B0155	9	2	1177	4	2	0	2	1135	?	?	2n = 2x
Alcantarea imperialis (Carrière) Harms	B0001	2	2	1177	5	1	1	2	1135	1134!	1135!	2n = 2x
Alcantarea imperialis (Carrière) Harms	B0135	0	1	1177	0	0	1	1	1135	1135	1135	2n = 2x
Alcantarea imperialis (Carrière) Harms	B0154	4	2	1177	0	2	1	2	1137	?	?	2n = 2x
Alcantarea nahoumii (Leme) J.R. Grant	B0156	0	1	1177	0	0	1	1	1133	1133	1133	2n = 2x
Alcantarea nevaresii Leme	B0163	1	2	1177	0	0	0	1	1134	1134	1134	2n = 2x
Alcantarea nevaresii Leme	B0169	1	2	1177	2	0	1	2	1134	1134	1134	2n = 2x

		PHYC			*PRK*							
Taxon	**DNA no.**	**SNPs**	**Geno types**	**Length**	**SNPs**	**Indels**	**SSR**	**Geno types**	**Consensus length**	**Allele 1 length (a1)**	**Allele 2 length (a2)**	**postulated ploidy level**
Alcantarea odorata (Leme) J.R. Grant	B0157	0	1	1177	0	0	0	1	1133	1133	1133	2n = 2x
Alcantarea regina (Vell.) Harms	B0136	0	1	1177	0	0	0	1	1134	1134	1134	2n = 2x
Alcantarea roberto-kautskyi Leme	B0158	2	2	1177	2	1	1	2	1140	1140	1138	2n = 2x
Alcantarea sp.	B0160	0	1	1177	1	1	1	2	1134	1134	1133	2n = 2x
Alcantarea sp.	B0168	5	2	1177	0	0	1	1	1133	1133	1133	2n = 2x
Vriesea Lindl.												
Vriesea correia-araujoi E. Pereira & I.A. Penna	B0095	0	1	1177	12	3	0	2	1118	1118	1116	2n = 2x
Vriesea croceana Leme & G.K. Br.	B0802	1	2	1177	7	0	0	2	1134	1134	1134	2n = 2x
Vriesea aff. *croceana* Leme & G.K. Br.	B0801	0	1	1177	0	0	1	1	1133	1133	1133	2n = 2x
Vriesea erythrodactylon (E. Morren) E. Morren ex Mez	B0309	0	1	1177	0	0	0	1	1133	1133	1133	2n = 2x
Vriesea flava A.F. Costa, H. Luther & Wand.	B0322	4	2	1180	0	0	0	1	999	999	999	2n = 2x
Vriesea jonghei (K. Koch) E. Morren	B0065	2	2	1177	3	2	1	2	1115	1115	1112	2n = 2x
Vriesea longicaulis (Baker) Mez	B0290	1	2	1177	7	2	1	2	1117	1117	1115	2n = 2x
Vriesea maxoniana (L.B. Sm.) L.B. Sm.	B0490	0	1	1180	0	0	0	1	1135	1135	1135	2n = 2x
Vriesea neoglutinosa Mez	B0311	7	2	1177	8	0	0	2	1121	1121	1121	2n = 2x
Vriesea pabstii McWill. & L.B. Sm.	B0357	0	1	1177	0	0	0	1	1115	1115	1115	2n = 2x
Vriesea platynema Gaudich.	B0146	0	1	1183	0	0	0	1	1135	1135	1135	2n = 2x
Vriesea plurifolia Leme	B0376	0	1	1177	0	0	1	1	1132	1132	1132	2n = 2x
Vriesea procera var. *tenuis* L.B. Sm.	B0296	0	1	1180	0	0	0	1	1138	1138	1138	2n = 2x
Vriesea psittacina (Hook.) Lindl.	B0020	6	2	1177	7	5	0	2	n.u.	1067!	999!	2n = 2x
Vriesea roethii W. Weber	B0511	1	2	1177	2	0	1	2	1117	1117	1117	2n = 2x
Vriesea ruschii L.B. Sm.	B0719	5	2	1177	11	0	0	2	1116	1116	1116	2n = 2x
Vriesea saundersii (Carrière) E. Morren ex Mez	B0004	0	1	1177	0	0	0	1	1133	1133	1133	2n = 2x
Vriesea scalaris E. Morren	B0147	0	1	1177	0	0	0	1	1027	1027	1027	2n = 2x
Vriesea simplex (Vell.) Beer	B0505	4	2	1177	5	1	0	2	1027	1027	1026	2n = 2x
Vriesea sparsiflora L.B. Sm.	B0484	2	2	1177	0	0	0	1	1115	1115	1115	2n = 2x
Vriesea sucrei L.B. Sm. & Read	B0302	1	2	1177	0	0	0	1	1024	1024	1024	2n = 2x
Vriesea unilateralis (Baker) Mez	B0316	2	2	1177	4	0	0	2	1117	1117	1117	2n = 2x

Appendix

Abstracts 2005–2012 (Conferences)
Oral presentations and posters

Botany 2012

Adaptive radiation, correlated evolution, and determinants of net diversification rates in Bromeliaceae: Test of an a priori model

Thomas J. Givnish [1], **Michael H.J. Barfuss** [2], Benjamin Van Ee [3], Ricarda Riina [4], Katharina Schulte [5], Ralf Horres [6], Philip A. Gonsiska [1], Rachel S. Jabaily [1], Darren M. Crayn [5], J. Andrew C. Smith [7], Klaus Winter [8], Gregory K. Brown [9], Timothy M. Evans [10], Bruce K. Holst [11], Harry E. Luther [11], Walter Till [2], Georg Zizka [12], Paul E. Berry [4] & Kenneth .J. Sytsma [1]

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Oral presentation: (638) in: *Botany 2012: The Next Generation* (abstracts). Columbus, Ohio: Greater Columbus Convention Center, 7–11 July 2012.

Abstract

We present an integrative model predicting associations among epiphytism, the tank habit, entangling seeds, C3 vs. CAM photosynthesis, avian pollinators, life in fertile, moist montane habitats, and species richness and net rates of species diversification in the monocot family Bromeliaceae. We test the predictions of this model by overlaying individual character-states on a molecular phylogeny, relating evolutionary shifts to time and reconstructed shifts in geographic distribution; by quantifying patterns of correlated and contingent evolution among pairs of traits; and by analyzing the apparent impact of individual traits on diversification within subfamilies. All patterns of correlated evolution among pairs of traits and environmental conditions predicted by our model were significant. The pattern and timing of shifts in phenotype and expansion of distributions outside the Guayana Shield also generally accorded with the model's predictions. Patterns of contingent evolution were largely consistent with the model. Species richness and net rates of species diversification were most closely tied to life in fertile, moist, geographically extensive cordilleras, with weaker ties to epiphytism, avian pollination, and the tank habit. The highest rates of diversification were seen in the core tillandsioids, associated with the Andes, and especially the tank-epiphyte clade of bromelioids, associated with the Serra do Mar and nearby ranges. Six adaptive radiations and associated speciation - including one clade of CAM epiphytes, one of predominantly C3 epiphytes, three of CAM terrestrials in arid habitats (in Central America, high elevations in the Andes and low elevations in the Brazilian Shield), and one of C3 terrestrials in rain- and cloud-forest understories - account for > 80% of total species number in the family. This integrative study is among the first to test a priori hypotheses about the relationships among phylogeny, phenotypic evolution, geographic spread, and net species diversification.

XVIII IBC 2011

Systematics, evolution, and phylogeography of *Tillandsia* (Bromeliaceae) and related genera

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Oral presentation: P. 189 (SYM004) in: *XVIII IBC 2011: XVIII International Botanical Congress* (abstracts). Melbourne, Australia: Melbourne Congress and Exhibition Centre, 23–30 July 2011.

Abstract

Tillandsia L. is the largest genus within Bromeliaceae comprising more than 660 accepted species in six recognized subgenera (*Tillandsia* subgen. *Allardtia*, *T*. subgen. *Anoplophytum*, *T*. subgen. *Diaphoranthema*, *T*. subgen. *Phytarrhiza*, *T*. subgen. *Pseudalcantarea*, *T*. subgen. *Tillandsia*). Eight additional genera have been associated with *Tillandsia* in subfamily Tillandsioideae, i.e., *Alcantarea*, *Catopsis*, *Glomeropitcairnia*, *Guzmania*, *Mezobromelia*, *Racinaea*, *Vriesea*, and *Werauhia*. The segregation of a group of *Tillandsia* species occurring in Mexico (*Viridantha*) and the transfer of xerophytic *Vriesea* species to *Tillandsia* is not accepted by all bromeliad researchers based on long-established morphological characterizations of traditionally accepted genera. Habit shifts from phytotelms to extreme xerophytes, life form transitions from terrestrials to epiphytes and lithophytes, shifts in pollination syndromes, and seed and stigma morphology are key events and characters for the evolution and systematics of genera and infrageneric units, but they can only be interpreted in the context of DNA sequence data, since some of these features have evolved independently within different phylogenetic lineages. Therefore we conducted DNA sequence analyses from nuclear genes (i.e., *PRK* and *PHYC*, totaling about 2500 bp) and published chloroplast markers (with about 6500 bp) in combination with a reevaluation of morphological characters. Based on these results three new genera with a distinctive stigma and seed morphology are segregated from *Tillandsia*, i.e., *Josemania*, *Lemeltonia*, and *Rothowia*. Species of all three belonged to the former subgenus *Phytarrhiza*, which turned out to be highly polyphyletic. Circumscription of the remaining *Tillandsia* subgenera also changes significantly and new infrageneric taxa and groups emerge (e.g., *T*. subgen. *Pseudovriesea*, *T*. subgen. *Viridantha*). DNA data suggest that the subfamily had its origin in the geologically old parts of northern South America. From there the two earliest diverging lineages migrated into the Caribbean (*Catopsis*, *Glomeropitcairnia*) with some taxa extending also into the Andes and into Central America (*Catopsis*). The next diverging clade (Vrieseeae) splits into two subgroups, one radiating into eastern Brazil (*Alcantarea*, *Vriesea*), the other spreading into the Andes (*Cipuropsis*, *Werauhia*). Within Tillandsieae the earliest lineage is *Guzmania* with a predominately Andean distribution, but also extending into Central America and the Caribbean. Early diverging taxa of the core Tillandsieae have their current distribution mainly in the Andes of northern Peru, Ecuador and Colombia, which seems to be the ancient area for the whole *Tillandsia* s.l. complex. *Tillandsia* s.str. has two centers of diversity, one in the Northern and Central Andes, the other in the mountain systems of Northern Central America, which proves to have reached Mexico and adjacent areas during at least three independent colonization events from the South (*T*. subgen. *Pseudalcantarea*, *T*. subgen. *Tillandsia* p.p., and *T*. subgen. *Viridantha* p.p.).

XVIII IBC 2011

New taxonomic implications in Tillandsioideae (Bromeliaceae) based on DNA data and morphology

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Poster: Pp. 568–569 (P0730) in: *XVIII IBC 2011: XVIII International Botanical Congress* (abstracts). Melbourne, Australia: Melbourne Congress and Exhibition Centre, 23–30 July 2011.

Abstract

In order to establish a natural and stable classification system for Tillandsioideae we conducted phylogenetic analyses using newly generated single-copy nDNA sequences (i.e., *PHYC* and *PRK*) and published cpDNA data (i.e., *atpB-rbcL* spacer, *matK*, *rbcL*, partial *rbcL-accD* spacer, *rps16* intron, partial *trnK* intron, *trnL* intron and *trnL-trnF* spacer). Derived phylogenetic units were then characterized using previously used, neglected, and new morphological characters. A new classification for Tillandsioideae is urgently needed, since modern bromeliad taxonomists rely on a more than 30 years old monograph. Subsequent taxonomic changes were only partly summarized in Floras or recent taxonomic treatments of certain groups. Our results highlight the presence of two new subtribes (Cipuropsidinae, Vrieseinae), three new genera (*Josemania*, *Lemeltonia*, *Rothowia*), and three new subgenera (*Racinaea* subg. *Pseudophytarrhiza*, *T*. subg. *Viridantha*, and *T*. subg. *Pseudovriesea*). Beside species from newly erected taxa, several others are also reclassified where initial placement was controversial among bromeliad researchers. Parsimony analysis revealed the following, mostly well-supported general branching pattern: ((*Glomeropitcairnia*, *Catopsis*) (((*Alcantarea*, *Vriesea*) (*Werauhia*, *Cipuropsis–Mezobromelia* clade)) (*Guzmania*, ((*Josemania*, *Racinaea*) (*Rothowia*, (*Lemeltonia*, *Tillandsia*); Bayesian analysis in contrast revealed a slightly different branching pattern within core Tillandsieae: (*Guzmania*, ((*Josemania*, *Rothowia*) (*Racinaea* (*Lemeltonia*, *Tillandsia*))). Relationships of subgenera and informal clades within Tillandsia are mostly unsupported, but most clades themselves receive good statistical support. Although much progress has been achieved in exploring phylogenetic relationships of Tillandsioideae by combining both cpDNA and nDNA sequence data, a final and conclusive classification for the whole subfamily cannot be presented here. The most critical groups that remain to be solved using new molecular markers are the *Cipuropsis–Mezobromelia* alliance and the genus *Tillandsia*.

XVIII IBC 2011

Origin, phylogeny, adaptive radiation, and geographic diversification of Bromeliaceae

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Oral presentation: Pp. 186–187 (SYM004) in: *XVIII IBC 2011: XVIII International Botanical Congress* (abstracts). Melbourne, Australia: Melbourne Congress and Exhibition Centre, 23–30 July 2011.

Abstract

Sequence variation in eight rapidly evolving plastid regions for 90 bromeliad species from 45 of 56 described genera confirm that the eight bromeliad subfamilies are related to each other in ladder-like fashion: (Brocchinoidieae, (Lindmanioideae, (Tillandsioideae, (Hechtioideae, (Navioideae, (Pitcairnioideae, (Puyoideae, Bromelioideae). Puya shows a basal split between species found primarily at higher elevations in the Andes and those found near sea level in Chile. The earliest-divergent bromelioids are mostly restricted to the southern Andes and Chilean coast. We calibrated this phylogeny against the ages of fossil monocots using penalized likelihood, and assessed patterns of biogeographic spread using maximum parsimony, Bayesian inference, and S-DIVA. Bromeliads appear to have arisen in the Guayana Shield roughly 100 Mya, spread centrifugally in the New World, and reached tropical West Africa via long-distance dispersal 9.2 Mya. Modern lineages began to diverge from each other 19 Mya, with invasions of drier or higher peripheral areas in Central America and northern South America beginning 16–13 Mya, coincident with a major adaptive radiation – the 'bromeliad revolution' – involving the repeated evolution of epiphytism, the tank habit, CAM photosynthesis, and avian pollination, as well as several features of leaf and trichome anatomy, and an accelerated pace of species diversification. This revolution coincided with the uplift of the northern Andes and its invasion by epiphytic tillandsioids and by ancestors of the pitcairnioids, puyoids, and bromelioids. Bromelioids invaded the Serra do Mar and nearby mountains in southeastern Brazil, most likely from southern Chile, starting 9.1 Mya. A major radiation of epiphytic bromelioids in Brazil began 5.7 Mya. Calculations of net rates of species diversification for subfamilies or clades of similar rank range from 0.16 sp sp^{-1} My^{-1} in brocchinioids to 1.12 sp sp^{-1} My^{-1} in the tank-epiphytic bromelioids. Acquisition of the epiphytic habit and related traits appears to have accelerated net species diversification by favoring seed traits that increased attachment to epiphytic perches, and coincidentally increased the ability of tillandsioids and bromelioids to colonize extensive montane regions in the Andes and Central America, and in the Serra do Mar and nearby mountains in coastal Brazil, permitting geographic speciation to proceed in massively parallel fashion as epiphytes occupied a cloud-forest landscape dissected by numerous drier, lower valleys that could act as extrinsic barriers to gene flow. Avian pollination (mainly by hummingbirds) evolved at least twice, coincident with the invasion of cool, wet montane habitats. Net diversification rates were significantly higher in hummingbird-pollinated clades, perhaps reflecting the rise of gullet-shaped flowers adapted to such pollinators and the resulting opportunity for rapid partitioning of another rapidly speciating montane clade based on differences in bill length. Entomophily is the ancestral condition in bromeliads, and bat pollination appears to have evolved several times from hummingbird pollination; chiropterophily is associated with warmer and/or drier conditions then ornithophily.

BioSystematics 2011

Conflicting phylogenetic signal within the nuclear marker *PRK* highlights the importance of hybridization events in the diversification of Bromeliaceae

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Poster: Pp. 252–253 (II-74) in: *BioSystematics Berlin 2011: 7th International Congress of Systematic and Evolutionary Biology (ICSEB VII), 12th Annual Meeting of the Society of Biological Systematics (Gesellschaft für Biologische Systematik, GfBS), 20th International Symposium "Biodiversity and Evolutionary Biology" of the German Botanical Society (DBG)* (abstracts). Berlin, Germany: 21–27 February 2011.

Abstract

The family Bromeliaceae includes 57 genera and over 1700 species which have a predominantly Neotropical distribution (1 species in West Africa), and underwent several major radiation events over the past 20 million years. Despite the remarkable morphological diversity within the family only little variation is found in plastid DNA sequences, severely hampering the inference of interspecific relationships based on these markers. Nuclear markers generally yield a higher proportion of informative sites compared to plastid sequences, but are more difficult to amplify and problems in distinguishing paralogous and orthologous sequences may arise. We produced nuclear (PRK) and plastid alignments for representatives of the subfamilies Bromelioideae and Pitcairnioideae s.str. (with focus on the genus *Fosterella*) and investigated their contrasting evolutionary histories. Here we show that plastid and nuclear phylogenies yield incongruent topologies and that complex patterns within the nuclear phylogenetic reconstructions based on the *PRK* dataset are best explained with the occurrence of paralogous copies but also with past hybridization events within Bromelioideae as well as within *Fosterella*. The importance of hybridization in the rapid radiation of Bromeliaceae is discussed as well as potential strategies to extract phylogenetic information from these and similar data sets.

Systematics 2009

The genus *Deuterocohnia* Mez (Bromeliaceae): Conflicting data in phylogenetic analysis

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Oral presentation: P. 118 in: *Systematics 2009, 7th Biennial Conference of the Systematics Association* (abstracts). Leiden, The Netherlands: National Herbarium of The Netherlands and National Museum of Natural History Naturalis, Leiden University Medical Centre, 10–14 August 2009.

Systematics 2009

Phylogenetic utility of the nuclear marker *PRK* on a low taxonomic level: A case study in the genus *Fosterella* (Bromeliaceae)

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Oral presentation: P. 118 in: *Systematics 2009: 7th Biennial Conference of the Systematics Association* (abstracts). Leiden, The Netherlands: National Herbarium of The Netherlands and National Museum of Natural History Naturalis, Leiden University Medical Centre, 10–14 August 2009.

Botany & Mycology 2009

Classification, adaptive radiation, and geographic diversification in Bromeliaceae: Insights from a new multi-locus phylogeny

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Oral presentation: (753) In: *Botany & Mycology 2009* (abstracts). Snowbird, Utah, USA: Snowbird Center, 25–29 July 2009.

Abstract

The slow rate of molecular evolution in Bromeliaceae, frequent morphological homoplasy, and extensive molecular and morphological divergence from the families most closely to it have hampered progress toward an understanding of evolutionary relationships within the family. Here we present a molecular phylogeny based on more than 9500 aligned bases from eight rapidly genes and spacers, for 90 ingroup taxa and three outgroups. The eight-subfamily classification recently advanced by Givnish et al. is supported. Brocchinioideae is sister to all other extant bromeliads; Lindmanioideae, also endemic to the Guayana Shield, diverged next, followed by Tillandsioideae. It appears that xeromorphic Hechtioideae diverged from the bromeliad spine next, followed by Navioideae (endemic to the Guayana Shield, with one species on the Brazilian Shield), Pitcairnioideae, with Puyoideae and Bromelioideae being sister to each other. Calibration of the bromeliad molecular tree against dates corresponding to non-bromeliad fossils indicates that bromeliads began to divergence from other monocots 70 Mya, and that the extant bromeliad genera began to diverge from each other only in the last 19 My. Fifty-one million years between the origin of bromeliads and initial divergence of surviving lineages helps explain the difficulty in identifying their closest relatives. Extant species of *Brocchinia* began to diverge from each other about 17 Mya, before almost any other genera began to diverge, helping explain why *Brocchinia* shows such a wide range of adaptive types. The pace of diversification accelerated greatly 13 Mya, coincident with the rise of several morphological and physiological adaptations to dry or epiphytic conditions arose, and invasion of areas peripheral to the Guayana Shield. This “bromeliad revolution” corresponds to the time of multiple origins of CAM photosynthesis, epiphytism, bird pollination, tank habit (following its initial origin in *Brocchinia*), and absorptive trichomes. A new model for determinants of bromeliad diversity is presented and discussed.

Monocots IV

Molecular phylogenetics of *Tillandsia* (Bromeliaceae) and related genera

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Oral presentation: In: *Monocots IV: The 4th international conference on the comparative biology of the monocotyledons & the 5th international symposium on grass systematics and evolution* (abstracts). Copenhagen, Denmark: University of Copenhagen, H.C. Ørsted Institute, 11–15 August 2008.

Abstract

Tillandsia is the most species-rich genus of subfamily Tillandsioideae (Bromeliaceae). Its traditional delimitation and internal classification are the subjects of much debate and conflict with recent molecular studies. Previously investigated plastid regions have shown little variation across Tillandsioideae, due to rapid radiation and subsequent genetic isolation/speciation. Until now it has not been possible to unambiguously infer phylogenetic relationships within the core Tillandsioideae, especially the *Tillandsia* s. l. (incl. *Racinaea* and *Viridantha*) and *Vriesea* s. l. (incl. *Alcantarea* and *Werauhia*) backbones. In this study we have analysed additional chloroplast and nuclear DNA markers to obtain more phylogenetic information, with the particular aim of resolving these backbones better. Results from the difficult ITS (Internal transcribed spacer) region show ITS 1 to be highly variable, but the ITS region as a whole contains only little phylogenetic signal. Therefore, sequences from the single-copy nuclear genes *PRK* (Phosphoribulokinase) and *PHYC* (Phytochrome C) are here combined with ITS and chloroplast sequence data to provide a well-supported molecular phylogeny. Only now we can propose a novel classification for *Tillandsia* and its closely related genera that were only partially reflected by previous chloroplast studies.

Monocots IV

Adaptive radiation and diversification in Bromeliaceae: Inferences from a new multigene phylogeny

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Oral presentation: In: *Monocots IV: The 4th international conference on the comparative biology of the monocotyledons & the 5th international symposium on grass systematics and evolution* (abstracts). Copenhagen, Denmark: University of Copenhagen, H.C. Ørsted Institute, 11–15 August 2008.

Abstract

Bromeliads show an unusually slow rate of molecular evolution, substantial homoplasy in morphological character-states, and substantial molecular and morphological divergence from other monocots, making inference of relationships within the family difficult. Here we present the most comprehensive analysis of bromeliad phylogeny to date, based on sequencing three rapidly evolving plastid genes (*matK*, *ndhF*, *rps16*) and three rapidly evolving plastid spacers (*psbA-trnH*, *rbcL-atpB*, *trnL-trnF*) across nearly 100 bromeliad taxa and outgroups drawn from six other families of Poales. We use these data to test the monophyly and recently proposed relationships among the eight subfamilies of Bromeliaceae; trace the evolution of several characters crucial for the evolution of the epiphytic habit under parsimony, maximum likelihood, and Bayesian frameworks; infer the broad-scale geographic patterns of geographic diversification within the family; and produce a calibrated chronology of evolution within the family. Together, these findings allow us to identify the timing of the "bromeliad revolution", during which several traits crucial to extensive speciation within the family apparently arose, accompanying its breakout from the exceedingly humid, infertile environments of the Guayana Shield.

Systematics 2008

The backbone problem: Hunting for informative characters in DNA sequences of Tillandsioideae (Bromeliaceae) for reconstructing a well-resolved phylogeny

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Oral presentation: P. 356 in: Gradstein, R. & al. (eds.), *Systematics 2008: 10th Annual Meeting of the Gesellschaft für Biologische Systematik, 18th International Symposium "Biodiversity and Evolutionary Biology" of the German Botanical Society (DBG)* (abstracts). Göttingen, Germany: University of Göttingen, 7–11 April 2008.

Abstract

Plastid Genomes have shown to be very uniform in recently diverged plant groups (like in Bromeliaceae) mainly caused by rapid radiation (and subsequent quick speciation / isolation). In addition to the less variable chloroplast regions which we analysed in our previous studies, we sequenced another set of cpDNA markers, which are supposed to be phylogenetically much more informative. Nevertheless, avoiding results of still insufficiently resolved phylogenies derived from this additional chloroplast data we also established primers for nuclear DNA regions, especially with the target of getting well resolved backbones in our trees. Attempts of several research groups to amplify the nrDNA region ITS (Internal Transcribed Spacer) in Bromeliaceae failed so far, because of very strong bounded secondary structures caused by repetitive GC-rich motives in the ITS 1 region. While we were able to successfully amplify and sequence over this difficult region for a set of species of Bromeliaceae by modifying PCR programs and sequencing chemistries, results of sequence analysis again showed very poor amounts of phylogenetically informative characters among taxa, having a similar substitution rate as it can be found in the investigated chloroplast regions (which is in contrast to many other plant groups). All resulting trees (even from combined analyses) did not yet allow an unambiguous interpretation of phylogenetic relationships of the whole subfamily Tillandsioideae; especially not at the backbone of very species-rich genera like *Tillandsia* and close relatives (*Racinaea*, *Viridantha*), and *Vriesea* and associates (*Alcantarea*, *Werauhia*). Therefore, sequences (including exons as well as introns) of the assumed single-copy nDNA genes *PRK* (Phosphoribulokinase), *PHYC* (Phytochrom C) and *MS* (Malate Synthase) have been analysed, and results compared with phylogenies derived from ITS and more than 10 different plastid regions; this nuclear markers seem to be much more promising in giving higher phylogenetic signals per sequenced base pair than any of the other previously investigated DNA regions does.

XVII IBC 2005

Phylogenetics of subfamily Tillandsioideae (Bromeliaceae): A comparison of plastid and nuclear DNA sequence with morphological data

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Oral presentation: P. 150 (9.7.3.) in: *XVII IBC 2005: XVII International Botanical Congress* (abstracts). Vienna, Austria: Austria Center Vienna, 17–23 July 2005.

Abstract

Part of the low-copy nuclear gene phosphoribulokinase (*PRK*) and a multicopy nuclear rDNA spacer region (ITS) were used to clarify relationships of subfamily Tillandsioideae (Bromeliaceae). These data are compared with the available cpDNA sequences of coding *rbcL* and *matK* and noncoding *trnL* intron, *trnL-F* intergenic spacer, *atpB-rbcL* intergenic spacer, *rps16* intron, and partial 5' and 3' *trnK* intron. We added taxa of *Tillandsia*, *Guzmania* and *Vriesea* to get better resolution within these genetically convergent genera. Molecular phylogenetic data is compared with various morphological characters to test their usefulness for a new classification of Tillandsioideae. These characters are optimised onto a strict consensus tree of the combined matrices of plastid and nuclear DNA sequences. Preference has been given to characters likely to be little affected by adaptive constraints, i.e., pollen, stigma, ovules, anther, seed, and nectary morphology.

Conclusions

The current study demonstrates that the analyses of additional plastid and nuclear DNA markers are able to provide better resolution and support in phylogenetic trees of Bromeliaceae. The inclusion of more taxa significantly improved the tree topologies, especially where more distantly related taxa were sampled in order to break long branches, while sampling of lineages which were not included before tend to decrease the support for certain clades. However, in all cases several unknown relationships could be resolved.

Analyses of plastid DNA sequences including more markers and more species clarified several uncertain relationships which existed among subfamilies of Bromeliaceae. The eight subfamilies are confirmed, but uncertainty about a monophyletic origin of subfamily Puyoideae persists. Previously proposed hypotheses concerning biogeography, origin and age of Bromeliaceae could be re-analysed and refined with the enlarged data set. Rapid radiation and relatively recent diversification within the last 8.7–4 My are most likely the explanation for the low sequence divergence.

The investigation of the low-copy nuclear gene *PRK* has provided useful insights into phylogenetic relationships of Bromelioideae. The amplified region is at least 3-times more variable than any plastid regions, thus making *PRK* suitable for phylogenetic reconstructions. However, the amplified fragment alone did not provide sufficient information for resolving relationships completely, only the combined analysis with plastid markers clarified several previously unresolved relationships at deeper nodes (“basal bromelioids”, “eu-bromelioids”). Genera within core bromelioids remain problematic. Most parsimonious reconstructions for tank habit and the sepal symmetry revealed strong evolutionary significance and indicated diagnostic utility for future classifications.

The comparative studies of nuclear DNA sequences within Tillandsioideae showed that some nuclear markers are able to provide more information and a higher degree of resolution in phylogenetic trees than plastid markers. However, their utility cannot be measured only by PICs, but depends also on methodological challenges. Nuclear ITS nrDNA is not recommended for phylogenetic investigations of Bromeliaceae due to the presence of strong secondary structures, which create problems in amplification and sequencing, as well as few PICs to resolve deeper nodes. Some other amplified fragments of genes were to short and showed few potentially PICs. The selected nuclear DNA regions of *PRK* and *PHYC* are able to provide reasonable support in some parts of phylogenetic trees, but several relationships still remain unresolved. So far, the search for “the ideal” phylogenetic marker for Tillandsioideae continues and perhaps it is impossible to find such a marker with standard PCR and Sanger-sequencing.

However, progress has been achieved in exploring phylogenetic relationships of Tillandsioideae by combining nuclear DNA sequence Data with already published plastid DNA sequence data. DNA results together with re-evaluated morphological characters allowed the recircumscription of existing genera and description of three new genera and subgenera, respectively. However, taxonomic problems in certain groups persist due to poor sampling, limited

resolution and variable morphology. To get a fully resolved picture of relationships within Tillandsioideae, more DNA data is needed as well as a careful revision of morphological characters which are already indicated to be useful to circumscribe taxonomic groups.

A major challenge that remains within Bromeliaceae is the great number of species, for which it is sometimes impossible to get material for both DNA and morphological investigations. Many species are not cultivated in *ex situ* collections, because either they are simply not available or require very special growing conditions. Several species were collected only once in the wild or are known from a single locality. If plant material is available, it is rather simple to generate additional DNA sequence data for phylogenetic studies, but collecting morphological characters is still challenging. Many useful characters are missing on herbarium specimens and flowering material is sometimes hardly available for certain species. Without having flowering or fruiting specimens (ideally both) in good conditions, a careful taxonomic revision is impossible. The future direction will be to establish improved international collaborations with taxonomists in the countries of origin of Bromeliaceae to access important plant material more easily.

The continuously improving next generation sequencing (NGS) technologies will play important roles in future phylogenetic investigations of Bromeliaceae. Sequencing and analysing of plastoms has already been successfully implemented for monocots; this will definitely help to clarify relationships and uncover the evolutionary history of the plastid genome in Bromeliaceae. The analysis of the entire 18S-5.8S-26S nrDNA repeat unit can also provide useful insights into the evolution of nrDNA in bromeliads and uncover the ecological significance of the strong secondary structure. NGS of nrDNA might also be useful to differentiate species and therefore could be a potential candidate marker for DNA barcoding. Furthermore, the possibility to gain huge amounts of allelic nuclear data will help to identify nuclear regions that are useful for inferring relationships and uncover hybridisation events.

This work aimed to provide a starting point for a biological classification of Bromeliaceae apart from the already well supported subfamilies. It intends to stimulate researchers interested in Bromeliaceae to focus on certain taxa, collect morphological information and apply newest sequencing technologies to answer several, to date unresolved evolutionary questions in Bromeliaceae.

Curriculum Vitae

Mag. Michael H.J. Barfuss

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Academic degree	Mag.rer.nat.
Date of birth, nationality	15.03.1977, Austria
Home address	Schillerstraße 12 Haus 12 2351 Wiener Neudorf, Austria
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Employment	
since 2006	Technical laboratory assistant, molecular systematics laboratory (lab management and sequencing technology); research associate, Department of Systematic and Evolutionary Botany, University of Vienna
Academic career	
2006–2012	Dr. student, Botany, University of Vienna, Austria
2006	Diplom (Mag.rer.nat), supervisors ao. Univ.-Prof. Dr. Rosabelle Samuel, o. Univ.-Prof. Dr. Tod Stuessy, final exam (Diplomprüfung) 25. August 2006, title of diploma thesis *Molecular studies in Bromeliaceae: Phylogenetic relationships in subfamily Tillandsioideae based on evidence from plastid sequences*
1996–2006	Diploma student, Biology (Botany), University of Vienna, Austria
1996	Qualification for university entrance (Matura) 3. June 1996, Höhere Bundeslehr- und Versuchsanstalt für Gartenbau, Vienna, Austria

Project leadership	
since 2011	*Evolution of the* Tillandsia capillaris *complex (Bromeliaceae): Adaptive radiation in a semi-arid environment?*, project leaders Dr. Jorge Chiapella (Argentinia), Ass.-Prof. Dr. Walter Till, Mag. Michael H.J. Barfuss (Austria) (BMWF, OeAD, WTZ)
since 2008	*Molecular Phylogeny of* Gymnocalycium *(Cactaceae)*, project leaders Mag. Michael. H.J. Barfuss, Ass.-Prof. Dr. Walter Till (GÖK, AGG)
2007–2008	*Evolution und Sekundärstruktur der nrDNS-Region ITS von* Tillandsia *s. l. (Bromeliaceae): Zusammenhänge zwischen Sequenz-Alignments, Phylogenie und ökologisch-adaptiver Variabilität*, project leaders Ass.-Prof. Dr. Walter Till, Mag. Michael. H.J. Barfuss (ÖAW, KIÖS no. 2007-02)
Project collaboration	
since 2012	*Phylogeny and evolution of Tillandsia subgenus Tillandsia (Bromeliaceae)*, project leaders Ass.-Prof. Dr. Walter Till, Mag. Michael H.J. Barfuss; postdoc Juan Pablo Pinzón
since 2003	*Phylogeny, adaptive radiation, and historical biogeography in Bromeliaceae*, The Bromeliad Phylogeny Group, group leader Prof. Dr. Thomas J. Givnish
2003	*Molecular phylogeny of Bromeliaceae subfamily Tillandsioideae: Insights from the nuclear and chloroplast genome*, project leader Ass.-Prof. Dr. Walter Till (ÖAW, KIÖS)
2002	*Molekulare Analyse der Verwandtschaftsbeziehungen innerhalb der Gattung Tillandsia (Bromeliaceae, Unterfamilie Tillandsioideae)*, project leader ao. Univ.-Prof. Dr. Rosabelle Samuel (Hochschuljubiläumsstiftung der Stadt Wien)
Project assistance	
since 2010	*Evolution and Biodiversity of New Caledonian* Diospyros *(Ebenaceae)*, project leader ao. Univ.-Prof. Dr. Rosabelle Samuel (FWF no. P22159)
2007–2011	*Taxonomische Überarbeitung der Gattung* Leontopodium, project leader o. Univ.-Prof. Dr. Hermann Stuppner (FWF no. P19480)
2006–2010	*Species delineation and autecology of* Spirogyra, project leader ao. Univ.-Prof. Mag. Dr. Michael Schagerl (FWF no. P18465)
2006–2010	*Molecular Phylogeny, genome size and chromosomal evolution in genus* Polystachya *Hooker (Orchidaceae)*, project leader ao. Univ.-Prof. Dr. Rosabelle Samuel (FWF no. P19108)
2005–2006	*Chromosomes and evolution of* Melampodium *(Asteraceae)*, project leader o. Univ.-Prof. Dr. Tod F. Stuessy (FWF no. P18201)

2004–2007	*Phylogeny and Historical Biogeography of Ebenaceae*, project leader ao. Univ.-Prof. Dr. Rosabelle Samuel (FWF no. P17094)
2002	*Phylogenie and historical biogeography of Phyllanthaceae*, project leader ao. Univ.-Prof. Dr. Rosabelle Samuel (FWF no. P15333)
2001–2002	*Intraspecific phylogeography of alpine plants*, project leader Univ.-Prof. Dr. Harald Niklfeld (FWF no. P13874)
2000–2002	*Generic delimitations and molecular phylogeny of subfamily Tillandsioideae (Bromeliaceae)*, project leader Ass.-Prof. Dr. Walter Till (FWF no. P13690)
Grants (donator)	
2011	*XVIII IBC 2011*, congress (University of Vienna)
2009	Jodrell Laboratory, Royal Botanic Gardens, Kew (Synthesys)
2008	*Systematics 2008*, conference (University of Vienna)
2008	*Monocots IV*, conference (ÖFB)
2003	*Monocots III*, conference (ÖFB)
Student tutorials	
2008–2009	Project practical course in DNA markers and chromosomes in plant systematics and evolutionary research
2005–2007	Practical course in population biology (AFLP, SSR)
2004–2010	Practical course in DNA sequencing analysis and molecular phylogeny
2001–2002	Practical course in enzyme analysis in plant systematics
2001	Plant systematic-morphological introductory excursions
2000	Plant systematic-morphological practical course
Teaching experience	
since 2012	Project practical course in molecular and karyological methods in evolution and ecology
since 2011	Project practical course in DNA barcoding - a new approach to species identification in ecology and biodiversity research
since 2010	Practical course in DNA sequencing analysis and molecular phylogeny
since 2010	Lectures in macromolecules and molecular phylogeny in plant systematics and evolutionary research
2010–2011	Project practical course in DNA markers and chromosomes in plant systematics and evolutionary research

International field trips	
2006	Ecuador
2006	USA (SW)
2002	Dominican Republic
2001	Borneo (Brunei Darussalam, Malaysia)
2000	USA (SW)
2000	Costa Rica
Invited talks	
2009	*Implications of five nuclear regions on the phylogenetic relationships of Tillandsioideae (Bromeliaceae)*; Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, UK
2006	*Tillandsien und verwandte Gattungen: Kann uns die DNA in der Systematik die fehlenden Antworten liefern? Ein Überblick über unseren derzeitigen Wissenstand*; Biologiezentrum Linz der OÖ. Landesmuseen, Linz, Austria
International conferences	
2011	*XVIII IBC 2011*, Melbourne, Australia; oral presentation, poster
2010	*Biodiversity and Evolutionary Biology*, Vienna, Austria
2008	*Monocots IV*, Copenhagen, Denmark; oral presentation
2008	*Sytematics 2008*, Göttingen, Germany; oral presentation
2007	*9. Jahrestagung der GfBS*, Vienna, Austria
2005	*XVII IBC 2005*, Vienna, Austria; oral presentation
2003	*Monocots III*, Claremont, California, USA; oral presentation
2001	*Systematics 2001*, London, UK; oral presentation
Scientific memberships	
Since 2006	American Society of Plant Taxonomists, ASPT
Since 2006	International Association for Plant Taxonomy, IAPT
Since 2005	Botanical Society of America, BSA

Special interests

All topics concerning the angiosperm families Bromeliaceae and Cactaceae; succulent plants; Asteraceae, Orchidaceae; special focus on molecular techniques (sequencing), (molecular) systematics, taxonomy, nomenclature, and field botany; plant cultivation and propagation, protection and preservation of endangered plant species (CITES).

Publications

00. **Barfuss, M.H.J., W. Till & R. Samuel.** In prep. A new classification of Bromeliaceae subfamily Tillandsioideae inferred from DNA sequences data of two genomes and morphology.
00. **Barfuss, M.H.J., R. Samuel, M.W. Chase & F. Forest.** In prep. Optimizing eight nuclear DNA markers for phylogenetic studies in recently diverged angiosperms: A case study in Bromeliaceae subfamily Tillandsioideae.
00. **Givnish, T.J., M.H.J. Barfuss, B. Van Ee, R. Riina, K. Schulte, R. Horres, P.A. Gonsiska, R.S. Jabaily, D.M. Crayn, J.A.C. Smith, K. Winter, G.K. Brown, T.M. Evans, B.K. Holst, H.E. Luther, W. Till, G. Zizka, P.E. Berry & K.J. Sytsma.** In prep. Adaptive radiation, correlated and contingent evolution, and net species diversification in Bromeliaceae.
00. **Sehr, E.M., M.H.J. Barfuss, G.E. Barboza, R. Samuel, E.A. Moscone & F. Ehrendorfer.** In prep. DNA sequences from the plastid genome and the single-copy nuclear gene *waxy* suggest two dysploid karyotype changes in the phylogeny of *Capsicum* (Solanaceae).
17. **Chen, C., M.H.J Barfuss, T. Pröschold & M. Schagerl.** 2012. Hidden genetic diversity in the green alga *Spirogyra* (Zygnematophyceae, Streptophyta). *B.M.C. Evolutionary Biology* (12): 77, doi 1186/1471-2148-12-77.
16. **Murillo-A., J., E. Ruiz-P., L.R. Landrum, T.F. Stuessy & M.H.J. Barfuss.** 2012. Phylogenetic relationships in *Myrceugenia* (Myrtaceae) based on plastid and nuclear DNA sequences. *Molecular Phylogenetics and Evolution*, 62 (2): 764–776.
15. **Demaio, P.H., M.H.J. Barfuss, R. Kiesling, W. Till & J. O. Chiapella.** 2011. Molecular phylogeny of *Gymnocalycium* (Cactaceae): Assessment of alternative infrageneric systems, a new subgenus, and trends in the evolution of the genus. *American Journal of Botany* 98 (11): 1841–1854.
14. **Givnish, T.J., M.H.J. Barfuss, B. Van Ee, R. Riina, K. Schulte, R. Horres, P.A. Gonsiska, R.S. Jabaily, D.M. Crayn, J.A.C. Smith, K. Winter, G.K. Brown, T.M. Evans, B.K. Holst, H.E. Luther, W. Till, G. Zizka, P.E. Berry & K.J. Sytsma.** 2011. Phylogeny, adaptive radiation, and historical biogeography in Bromeliaceae: Insights from an eight-locus plastid phylogeny. *American Journal of Botany* 98 (5): 872–895.
13. **Demaio, P.H., M.H.J. Barfuss, W. Till & J. O. Chiapella.** 2010. Phylogenetic relationships and infrageneric classification of the genus *Gymnocalycium*: Insights from molecular data. *Gymnocalycium* (Sonderausgabe 2010): 925–946.
12. **Russell, A., R. Samuel, V. Klejna, M.H.J. Barfuss, B. Rupp & M.W. Chase.** 2010. Reticulate evolution in diploid and tetraploid species of *Polystachya* (Orchidaceae) as shown by plastid DNA sequences and low-copy nuclear genes. *Annals of Botany* 106 (1): 37–56.
11. **Russell, A., R. Samuel, B. Rupp, M.H.J. Barfuss, M. Šafran, V. Besendorfer & M.W. Chase.** 2010. Phylogenetics and cytology of a pantropical orchid genus *Polystachya* (Polystachyinae, Vandeae, Orchidaceae): Evidence from plastid DNA sequence data. *Taxon* 59 (2): 389–404.
10. **Blöch, C., H. Weiss-Schneeweiss, G.M. Schneeweiss, M.H.J. Barfuss, C.A. Rebernig, J.L. Villaseñor & T.F. Stuessy.** 2009. Molecular phylogenetic analyses of nuclear and plastid DNA sequences support dysploid and polyploid chromosome number changes and reticulate evolution in the diversification of *Melampodium* (Millerieae, Asteraceae). *Molecular Phylogenetics and Evolution* 53 (1): 220–233.

9. **Duangjai, S., R. Samuel, J. Munzinger, F. Forest, B. Wallnöfer, M.H.J. Barfuss, G. Fischer & M.W. Chase.** 2009. A multi-locus plastid phylogenetic analysis of the pantropical genus *Diospyros* (Ebenaceae), with an emphasis on the radiation and biogeographic origins of the New Caledonian endemic species. *Molecular Phylogenetics and Evolution* 52 (3): 602–620.
8. **Gruenstaeudl, M., E. Urtubey, R.K. Jansen, R. Samuel, M.H.J. Barfuss & T. F. Stuessy.** 2009. Phylogeny of Barnadesioideae (Asteraceae) inferred from DNA sequence data and morphology. *Molecular Phylogenetics and Evolution* 51 (3): 572–587.
7. **Schulte, K., M.H.J. Barfuss & G. Zizka.** 2009. Phylogeny of Bromelioideae (Bromeliaceae) inferred from nuclear and plastid DNA loci reveals the evolution of the tank habit within the subfamily. *Molecular Phylogenetics and Evolution* 51 (2): 327–339.
6. **Möller, M., M. Pfosser, C.-G. Jang, V. Mayer, A. Clark, M.L. Hollingsworth, M.H.J. Barfuss, Y.-Z. Wang, M. Kiehn & A. Weber.** 2009. A preliminary phylogeny of the 'didymocarpoid Gesneriaceae' based on three molecular data sets: Incongruence with available tribal classifications. *American Journal of* Botany 96 (5): 989–1010.
5. **Till, W. & M.H.J. Barfuss.** 2006. Progress towards a new classification of Tillandsioideae. *Journal of the Bromeliad Society* 56 (6): 253–259.
4. **Barfuss, M.H.J., R. Samuel, W. Till & T.F. Stuessy.** 2005. Phylogenetic relationships in subfamily Tillandsioideae (Bromeliaceae) based on DNA sequence data from seven plastid regions. *American Journal of Botany* 92 (2): 337–351.
3. **Samuel, R, H. Kathriarachchi, P. Hoffmann, M.H.J. Barfuss, K.J. Wurdack, C.C. Davis & M.W. Chase.** 2005. Molecular phylogenetics of Phyllanthaceae: Evidence from plastid *matK* and nuclear *PHYC* sequences. *American Journal of Botany* 92 (1): 132–141.
2. **Barfuss, M.H.J., R. Samuel & W. Till.** 2004. Molecular phylogeny in subfamily Tillandsioideae (Bromeliaceae) based on six cpDNA Markers: An update. *Journal of the Bromeliad Society* 54 (1): 9–17, 48.
1. **Schönswetter, P., A. Tribsch, M.H.J. Barfuss & H. Niklfeld.** 2002. Several Pleistocene refugia detected in the high alpine plant *Phyteuma globulariifolium* Sternb. & Hoppe (Campanulaceae) in the European Alps. *Molecular Ecology* 11 (12): 2637–2647.

Abstracts (Presenter)

37. [Oral presentation] **Turner, B., J. Munzinger, S. Duangjai, M.H.J. Barfuss, B. Wallnöfer, O. Paun, M.W. Chase & R. Samuel.** 2012. Diversification of endemic New Caledonian *Diospyros* (Ebenaceae). P. 53 in: *Berichte des naturwissenschaftlich-medizinischen Vereins in Innsbruck, Supplementum 2020, 15. Treffen der Österreichischen Botanikerinnen und Botaniker* (abstracts). Innsbruck, Austria: Universität Innsbruck, Institut für Botanik, 27–29 September.
36. [Oral presentation] **Ehrendorfer, F., V. Vladimirov, J.-F. Manen & M.H.J. Barfuss.** 2012. Adaptive Radiation und weltweite Expansion der artenreichen Tribus Rubieae (Rubiaceae). P. 13 in: *Berichte des naturwissenschaftlich-medizinischen Vereins in Innsbruck, Supplementum 2020, 15. Treffen der Österreichischen Botanikerinnen und Botaniker* (abstracts). Innsbruck, Austria: Universität Innsbruck, Institut für Botanik, 27–29 September.

35. [Oral presentation] **Givnish, T.J., M.H.J. Barfuss, B. Van Ee, R. Riina, K. Schulte, R. Horres, P.A. Gonsiska, R.S. Jabaily, D.M. Crayn, J.A.C. Smith, K. Winter, G.K. Brown, T.M. Evans, B.K. Holst, H.E. Luther, W. Till, G. Zizka, P.E. Berry & K.J. Sytsma.** 2012. Adaptive radiation, correlated evolution, and determinants of net diversification rates in Bromeliaceae: Test of an a priori model. (638) in: *Botany 2012: The Next Generation* (abstracts). Columbus, Ohio: Greater Columbus Convention Center, 7–11 July.
34. [Oral presentation] **Barfuss, M.H.J., W. Till & R. Samuel.** 2011. Systematics, evolution, and phylogeography of *Tillandsia* (Bromeliaceae) and related genera. P. 189 (SYM004) in: *XVIII IBC 2011: XVIII International Botanical Congress* (abstracts). Melbourne, Australia: Melbourne Congress and Exhibition Centre, 23–30 July.
33. [Poster] **Barfuss, M.H.J., W. Till & R. Samuel.** 2011. New taxonomic implications in Tillandsioideae (Bromeliaceae) based on DNA data and morphology. Pp. 568–569 (P0730) in: *XVIII IBC 2011: XVIII International Botanical Congress* (abstracts). Melbourne, Australia: Melbourne Congress and Exhibition Centre, 23–30 July.
32. [Oral presentation] **Givnish, T.J., M.H.J. Barfuss, B. Van Ee, R. Riina, K. Schulte, R. Horres, P.A. Gonsiska, R.S. Jabaily, D.M. Crayn, J.A.C. Smith, K. Winter, B.K. Holst, H.E. Luther, W. Till, G. Zizka, P.E. Berry, Ann Arbor & K.J. Sytsma.** 2011. Origin, phylogeny, adaptive radiation, and geographic diversification of Bromeliaceae. Pp. 186–187 (SYM004) in: *XVIII IBC 2011: XVIII International Botanical Congress* (abstracts). Melbourne, Australia: Melbourne Congress and Exhibition Centre, 23–30 July.
31. [Oral presentation] **Ehrendorfer, F., V. Vladimirov & M.H.J. Barfuss.** 2011. Worldwide radiation, phylogeography and paraphyly of the speciose Rubiaceae-Rubieae. Pp. 253–254 (SYM094) in: *XVIII IBC 2011: XVIII International Botanical Congress* (abstracts). Melbourne, Australia: Melbourne Congress and Exhibition Centre, 23–30 July.
30. [Poster] **Russell, A., R. Samuel, M.H.J. Barfuss, B. Turner & M.W. Chase.** 2011. Evolutionary inference from multiple incongruent DNA data matrices: Reticulate evolution of *Polystachya* (Orchidaceae). P. 665 (P1042) in: *XVIII IBC 2011: XVIII International Botanical Congress* (abstracts). Melbourne, Australia: Melbourne Congress and Exhibition Centre, 23–30 July.
29. [Poster] **Samuel, R., B. Turner, S. Duangjai, J. Munzinger, B. Wallnöfer, M.W. Chase & M.H.J. Barfuss.** 2011. Origin and evolution of New Caledonian *Diospyros* (Ebenaceae): A phylogenetic approach. P. 635 (P0947) in: *XVIII IBC 2011: XVIII International Botanical Congress* (abstracts). Melbourne, Australia: Melbourne Congress and Exhibition Centre, 23–30 July.
28. [Poster] **Michalak, I., D. Silvestro, D. Brie, M.H.J. Barfuss, K. Schulte & G. Zizka.** 2011. Conflicting phylogenetic signal within the nuclear marker *PRK* highlights the importance of hybridization events in the diversification of Bromeliaceae. Pp. 252–253 (II-74) in: *BioSystematics Berlin 2011: 7th International Congress of Systematic and Evolutionary Biology (ICSEB VII), 12th Annual Meeting of the Society of Biological Systematics (Gesellschaft für Biologische Systematik, GfBS), 20th International Symposium "Biodiversity and Evolutionary Biology" of the German Botanical Society (DBG)* (abstracts). Berlin, Germany: 21–27 February.
27. [?] **Murillo-Aldana, J., E. Ruiz-P., L.R. Landrum, T.F. Stuessy & M.H.J. Barfuss.** 2010. Relaciones filogeneticas en *Myrceugenia*(Myrtaceae). In: *Latin American Botanical Congress*. La Serena, Chile: October.

26. [Oral presentation] **Russell, A., M.H.J. Barfuss, M.W. Chase & R. Samuel.** 2010. Reticulate evolution in diploid and tetraploid species of *Polystachya* (Orchidaceae) revealed by multiple nuclear and plastid loci. P. 55 in: Albach, D. & Greimler, J. (eds), *19th International Symposium "Biodiversity and Evolutionary Biology" of the German Botanical Society (DBG)* (abstracts). Vienna, Austria: University of Vienna, Faculty Centre of Biodiversity, 16–19 September.
25. [Poster] **Duangjai, S., R. Samuel, J. Munzinger, B. Wallnöfer, F. Forest, M.H.J. Barfuss &. M.W. Chase.** 2010. Biogeography origins and evolution of endemic species of *Diospyros* (Ebenaceae) from New Caledonia: A molecular phylogenetic perspective. P. 85 in: Albach, D. & Greimler, J. (eds), *19th International Symposium "Biodiversity and Evolutionary Biology" of the German Botanical Society (DBG)* (abstracts). Vienna, Austria: University of Vienna, Faculty Centre of Biodiversity, 16–19 September.
24. [Poster] **Stockenhuber, R.M., M.H.J. Barfuss, G. Cruz-Mazo & R. Samuel.** 2010. Molecular phylogeny of *Scorzoneroides*. P. 116 in: Albach, D. & Greimler, J. (eds), *19th International Symposium "Biodiversity and Evolutionary Biology" of the German Botanical Society (DBG)* (abstracts). Vienna, Austria: University of Vienna, Faculty Centre of Biodiversity, 16–19 September.
23. [?] **Russell, A., R. Samuel, V. Klejna, M.H.J. Barfuss, B. Rupp & M.W. Chase.** 2010. Analysis of multiple nuclear and plastid loci reveals reticulate evolution in diploid and tetraploid species of genus *Polystachya* (Orchidaceae). In: *New Frontiers in Plant Systematics and Evolution (NFPSE 2010)*. Beijing, China: 7–9 July.
22. [Oral presentation] **Schuetz, N., M.H.J. Barfuss, K. Weising & Georg Zizka.** 2009. The genus *Deuterocohnia* Mez (Bromeliaceae): Conflicting data in phylogenetic analysis. P. 118 in: *Systematics 2009, 7th Biennial Conference of the Systematics Association* (abstracts). Leiden, The Netherlands: National Herbarium of The Netherlands and National Museum of Natural History Naturalis, Leiden University Medical Centre, 10–14 August.
21. [Oral presentation] **Brie, D., K. Schulte, M.H.J. Barfuss & G. Zizka.** 2009. Phylogenetic utility of the nuclear marker *PRK* on a low taxonomic level: A case study in the genus *Fosterella* (Bromeliaceae). P. 33 in: *Systematics 2009, 7th Biennial Conference of the Systematics Association* (abstracts). Leiden, The Netherlands: National Herbarium of The Netherlands and National Museum of Natural History Naturalis, Leiden University Medical Centre, 10–14 August.
20. [Oral presentation] **Russell, A., R. Samuel, B. Rupp, M.H.J. Barfuss, M. Safran, V. Klejna & M.W. Chase.** 2009. Phylogeny, cytology and biogeography of the pantropical orchid genus *Polystachya*. P. 109 in: *Systematics 2009, 7th Biennial Conference of the Systematics Association*. Leiden, The Netherlands: National Herbarium of The Netherlands and National Museum of Natural History Naturalis, Leiden University Medical Centre, 10–14 August.
19. [Oral presentation] **Givnish, T.J., B. Van Ee, M.H.J. Barfuss, R. Riina, K. Schulte, R Horres, P.A. Gonsiska, R.S. Jabaily, D.M. Crayn, J.A.C. Smith, K. Winter, G.K. Brown, T.M. Evans, B.K. Holst, H.E. Luther, W. Till, G. Zizka, P.E. Berry & K.J. Sytsma.** 2009. Classification, adaptive radiation, and geographic diversification in Bromeliaceae: Insights from a new multi-locus phylogeny. (753) In: *Botany & Mycology 2009* (abstracts). Snowbird, Utah, USA: Snowbird Center, 25–29 July.

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